EVIDENTIARY APPENDIX

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Cited Authorities:

Miles Lab., Inc., v Shandon, Inc. 997 F.2nd 870, 875, 27 U.S.P.Q.2d 1123, 1126
(Fed. Cir. 1993)

copy attached

In re Moore, 169 U.S.P.Q. 236, 238 (CCPA 1971)

copy attached

Publication upon which the present application is based:

Rostovtsev, et al., *Angewandte Chemie Int. Ed., 41,* pages 2596-2599 (2002)

copy attached

Summary of 30 Laudatory References: copies attached

1. Accelerated Bioorthogonal Conjugation: A Practical Method for the Ligation of Diverse Functional Molecules to a Polyvalent Virus Scaffold

    "The azide group in particular has emerged as a very useful chemical handle for this purpose, participating in the Staudinger ligation with phosphines as well as the [3 + 2] cycloaddition reaction with strained and terminal alkynes. Under the influence of copper(I) catalysis, the latter process has permitted the selective modification of virus particles, enzymes, cells, and proteins from complex tissue lysates. The unreactive nature of both azides and alkynes with the functional groups of biomolecules, as well as their small size and therefore unobtrusive presence when appended to enzymatic substrates, makes improved methods for their ligation of interest for a wide range of biomolecular applications." Gupta S.S., et al., *Bioconjugate Chem.* (2005). **16**(6) 1572-1579 at page 1572, paragraph 1.

2. Synthesis of Symmetric and Unsymmetric Triazole Dendrimers via Dipolar Cycloaddition Reaction

    "Recently, the Cu(I)-catalyzed Huisgen [2 + 3] dipolar cycloaddition reaction between

an organic azide and a terminal alkyne, has attracted attention. The reaction, characterized by very high yields, mild and simple reaction conditions, oxygen and water tolerance, and simple product isolations, is highly chemoselective affording only the desired 1,2,3-triazole, even in the presence of a large variety of other functional groups." Lee, J.W. and Kim B-K., *Synthesis* (2006). 4 0165-0618 at 615, paragraph 1.

3. A Study of the Scope and Regioselectivity of the Ruthenium-Catalyzed [3+2]-Cycloaddition of Azides with Internal Alkynes

"As part of the work by the Sharpless group on so-called "click" reactions, whereby heteroatom links between molecules can be generated under mild conditions, it was found that cycloadditions of terminal alkynes with alkyl azides catalyzed by Cu(I) can be conducted at room temperature and are highly regioselective." Majireck, M.M. and Weinreb, S.M. *J. Org. Chem.* (2006) **71**(22) 8680-8683 at 8680, paragraph 1.

4. A Potentially Valuable Advance in the Synthesis of Carbhoydrate0Based Anticancer Vaccines though Extended Cycloaddition Chemistry.

"Notably, as practiced by Sharpless and associates, the cycloaddition reaction is highly chemoselective and tolerates a variety of functionality." Wan, Q. et al., *J. Org. Chem.* (2006) **71**(21) 8244-8249 at 8245, paragraph 5.

5. Combining ATRP and "Click" Chemistry: a Promising Platform toward Functional Biocompatible Polymers and Polymer Bioconjugates

"Recently, Sharpless et al. popularized in organic synthesis the 1,3-dipolar cycloaddition of azides and terminal alkynes, catalyzed by copper(I). Such reactions were proven to be very versatile, since they can be performed in high yield, in multiple solvents (including water) and in the presence of numerous other functional groups." Lutz, J-F. et al., *Macromolecules* (2006) **39**(19) 6376-6383 at 6376, paragraph 2.

6. Click Chemistry – What's in a Name? Triazole Synthesis and Beyond

"The observation that copper(I) salts promote faster (up to 10^7 times) and regiospecific couplings between terminal alkynes and azides allowed for the rapid development of this reaction; these results were reported independently by the groups of Sharpless and Meldal. Moreover, the cycloaddition is usually carried out at room temperature in aqueous media, and is compatible with most common function groups present in the molecules." Gil, M.V. et al., *Synthesis* (2007) **11** 1589-1620 at 1594, paragraph 2.

7. Multivalent, Bifunctional Dendrimers Prepared by Click Chemistry

"The key chemical transformation which allows simple and facile preparation of these dual-purpose, multifunctional materials is the copper(I)-catalyzed azide-alkyne cycloaddition, a premier 'click' reaction. The high efficiency of this process is exploited to couple the dendritic blocks together, while its tolerance of a wide variety of functional groups allows the introduction of reactive unites at the periphery without the use of protecting groups." Wu, P., et al., *Chem. Comm.* (2005) 5775-5777 at 5775, paragraph 1.

8. Cu^I-Catalyzed Alkyne-Azide "Click" Cycloadditions from a Mechanistic and Synthetic Perspective.

"Sharpless and co-workers have identified a number of reactions that meet the criteria for click chemistry, arguably the most powerful of which discovered to date is the CuI-catalyzed variant of the Huisgen 1,2-dipolar cycloaddition of azides and alkynes to afford 1,2,3-triazoles. As is not uncommon in organic synthesis, this reaction owes its usefulness in part to the ease with which azides and alkynes can be introduced into a molecule and their relative stability under a variety of conditions. Azides and alkynes are essentially inert to most biological and organic conditions, including highly functionalized biological molecules, molecular oxygen, water, and the majority of common reaction conditions in organic synthesis." Bock, V.D. et al., *Eur. J. Org. Chem.* (2006) 51-68 at 51, paragragh 2.

9. Efficient Synthesis of 1,4-disubsituted 1,2,3-triazoles in Ionic Liquid/water System.

"Sequentially, the copper(I)-catalyzed Huisgen cycloaddition reaction of azides and terminal alkynes has emerged as a novel alternative, and received much attention since its discovery. The methodology has found applications in drug discovery, bioconjugations and materials science. Exclusive regioselectivity, wide substrate scope and mild reaction conditions have made it the method of choice for making permanent connections by means of 1,4-disubstituted 1,2,3-triazoles. For instance, in the last few years, the copper(I) catalyzed Huisgen cycloaddition reaction has been widely used in multi-component reactions (MCRs) to prepare 1,2,3-triazoles with various functional groups." Zhao, Y-B. et al., *Tetrahedron Letters* (2006) 47 1545-1549 at 1545, paragraph 1.

10. Inhibitors of HIV-1 Protease by Using In Situ Click Chemistry

"Although the concept has been previously demonstrated by several researchers, the in situ click chemistry approach is unique in that it relies on the completely bio-orthogonal 1,3-dipolar cycloaddition of organic azides and alkynes." Whiting, M. et al., *Angew. Chem. Int. Ed.* (2006) **45** 1435-1439 at 1436, paragraph 2.

11. The Potential of Cycloaddition Reactions in the Synthesis of Dendritic Polymers

"One is the reaction of organic azides with alkynes forming a triazole ring, which experienced an enormous revival in many fields of polymer science after Sharpless and others pointed out that under suitable copper catalysis complete control of the regioselectivity can be achieved. This, in combination with easy accessibility of the monomers, very high yields, and the insensitivity of the cycloaddition reaction towards many functional groups which led to the term "click chemistry," is the base for the huge success of this CA reaction. Voit, B. *New J. Chem.* (2007) **31** 1139-1151 at 1144, paragraph 1.

12. 1,3-Dipolar Cycloadditions of Azides and Alkynes: A Universal Ligation Tool in Polymer and Materials Science

"However, in the presence of copper(I), which can bind to terminal alkynes, cycloaddition reactions are dramatically accelerated, regioselective, and highly efficient (yields are often above 95%). Moreover, the copper-catalyzed azide-alkyne cycloaddition (CuAAC) can be performed in various solvents (including water) and in the presence of numerous other functional groups." Lutz, J-F. *Angew. Chem. Int. Ed.* (2007) **46** 1018-1025 at 1018, page 2.

13. Heterogeneous Copper-in-Charcoal-Catalyzed Click Chemistry

"Noteworthy features of [click reactions catalyzed by Cu/C] include a) the regiochemistry (1,4-triazole) of addition follows that anticipated for such Cu-catalyzed reactions; b) outstanding functional group tolerance is observed under these heterogeneous conditions; c) yields are uniformly high; d) generally, reactions are complete within minutes; e) sterically demanding precursors are not precluded from participation; f) heteroaromatics coupled smoothly and g) high-molecular-weight adducts, such as the Triazole-containing steroidal derivative and the precursor to an analogue of coenzyme Q1 were both readily formed and isolated in good yield." Lipshutz, B.H. and Taft, B.R. *Angew. Chem. Int. Ed.* (2006) **45** 8235-8238 at 8236, paragraph 3.

14. Study of High Glass Transition Temperature Thermosets Made from the Copper(I)-Catalyzed Azide-Alkyne Cycloaddition Reaction

"The reaction is useful in biological settings for two main reasons: the azide and alkyne components are largely unreactive with biological molecules (and therefore selectively reactive with each other), and the product triazole can interact with biological structures in several noncovalent ways while being at the same time extraordinarily stable." Le Baut, N. et al., *Polymer* (2007) **48** 239-244 at 239, paragraph 2

15. Dressed-up Proteins

"To attach the carbohydrate PTM-mimic, van Kasteren *et al.* incorporated chemical groups (alkynes and azides) into the glycan and the protein. These groups react exceptionally well with each other under near-physiological conditions, but are indifferent to all other groups present." Grotenbreg, G. and Ploegh, H. *Nature* (2007) **446**(26) 993-995 at 994, paragraph 1.

16. A Chemoselective Approach for the Accelerated Synthesis of Well-Defined Dendritic Architectures

"A reaction that has gained tremendous attention since it was reported by Fokin and Sharpless in 2002 and which fulfills all the criteria for the construction of dendritic structure is the Cu catalyzed Click reaction of azides with terminal alkynes. This reaction is highly efficient and occurs under benign conditions in the presence of other reactive groups with no byproducts. Due to its orthogonal, robust nature, this example of Click chemistry has attracted significant attention in the materials science community, especially for the construction of complex polymeric materials." Antoni, P. et al., *Chem. Comm.* (2007) 2249-2251 at 2249, paragraph 3.

17. Bringing Efficiency to Materials Synthesis: The Philosophy of Click Chemistry

"The complete chemo- and regio-specificity, quantitative yields, and almost perfect fidelity in the presence of a wide variety of functional groups and reaction conditions that are the hallmarks of click reactions greatly enable the fabrication of well defined, multifunctional materials... The application of the copper(i)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction—so far, the best example of click chemistry—to materials and polymer synthesis has sparked a true re-invigoration of interest in this widely studied and critically important field." Hawker, C.J. et al. *Aust. J. Chem.* (2007) **60** 381-383 at 381, paragraphs 2-3.

18. Catalytic Azide-Alkyne Cycloaddition: Reactivity and Applications

"Around the same time, the concept of 'click chemistry' was introduced by Koblv, Finn, and Sharpless. It was defined as an efficient approach to the synthesis of diverse compounds based on a handful of 'near-perfect' (very selective, modular, high-yielding, wide in scope) carbon-heteroatom bond-forming reactions. The potential of organic azides as highly energetic, yet very selective, functional groups in organic synthesis was highlighted, and their dipolar cycloaddition with alkynes was placed among the top reactions fulfilling the click criteria." Wu, P. and Fokin, V. *Aldrichimicia ACTA* (2007) **40**(1) 7-17 at 7, paragraph 3.

19. Rapid Discovery and Structure-Activity Profiling of Novel Inhibitors of Huam Immunodeficiency Virus Type 1 Protease Enabled by the Copper(I)-Catalyzed Synthesis of 1,2,3-Triazoles and Their Further Functionalization

"The copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) has cemented its position at the heart of click chemistry due to its extremely broad scope, the orthogonality of the azide and alkyne functionalities to a diverse range of functional groups and reaction conditions, and the unique properties of the triazole products." Whiting, M. et al., *J. Med. Chem.* (2006) **49**(26) 7697-7710 at 7697, paragraph 2.

20. Synthesis of Saccharide-Terminated Poly (*ε*-caprolactone) via Micahel Addition and 'Click' Chemistry

"Recently, this reaction became an important method to modify polymers. 'Click' chemistry, originally developed for use in organic synthesis and chemical biology, is highly efficient and selective, and can be carried out in the presence of many functional groups with high yields. Recently, this 1,3-dipolar cycloaddition of azides and terminated alkynes by copper(I) catalyst has been widely used in the synthesis of end- or pendent functionalized polymers, new monomers and macromonomers, block copolymers, star, or cyclic polymers, and dendrimers." Xu, N., et al., *Macromol. Chem. Phys.* (2007) **208** 730-738 at 731, paragraph 3.

21. Click Chemistry in Materials Synthesis. II. Acid-Swellable Crosslinked Polymers Made by Copper-Catalyzed Azide-Alkyne Cycloaddition

"It was discovered in the early stages of our efforts to apply the copper(I)-catalyzed azide–alkyne cycloaddition click reaction to materials synthesis. The process is rapid and tolerant of a broad range of solvents and functional groups, making it of interest in the assembly of building blocks for organic, biological, and materials purposes." Li, C. and Finn, M.G., *J. of Polymer Science: Part A: Polymer Chem.* (2006) **44** 5513-5518 at 5513, paragraph 1.

22. Click Assembly of 1,2,3-Triazole-Linked Dendrimers, Including Ferrocenyl Dendrimers, Which Sense Both Oxo Anions and Metal Cations

"We have now assembled dendrimers linked by 1,2,3-triazole heterocycles by the Huisgen 1,3-dipolar cycloaddition between azides and alkynes, a reaction that has recently been greatly improved and defined by Sharpless as "click chemistry" because of its regioselectivity and catalytic course in the presence of CuI, its tolerance of a wide range of functionalities, and its high yields in water." Ornelas, C. et al., *Angew. Chem. Int. Ed.* (2007) **46** 872-877 at 873, paragraph 1.

23. 'Click' Bioconjugation of a Well-Defined Synthetic Polymer and Protein Transduction Domain

"Moreover, copper-catalyzed azide–alkyne cycloadditions (CuAAC) possess the important advantage of proceeding at room temperature, in relatively benign

solvents (including water), and in the presence of many other functional groups."
Lutz, J-F et al., *Aust. J. Chem.* (2007) **60** 410-413 at 410, paragraph 1.

24. 'Click' Chemistry in Polymer and Materials Science

"The basic process of the Huisgen 1,3-dipolar cycloaddition is depicted in Scheme 2, generating 1,4- and 1,5-triazoles, respectively. Nearly all functional groups are compatible with this process, except those that are a) either self reactive, or b) able to yield stable complexes with the CuI metal under catalyst deactivation." Binder, W.H. and Sachsenhofer, R. *Macromol. Rapid Commun.* (2007) **28** 15-54 at 17, paragraph 2.

25. The Allylic Azide Rearrangement: Achieving Selectivity

" Copper(I)-catalyzed cycloaddition with terminal alkynes, which results in 1,4-disubstituted 1,2,3-triazoles, is among the recent advances in the chemistry of organic azides.2 The rare chemical orthogonality of the azide and alkyne functionalities (that is, inertness to acidic and basic conditions) has enabled unique applications of this process in chemical biology, organic synthesis, and materials science." Feldman, A.K. et al., *J. Am. Chem. Soc.* (2005) **127** 13444-13445 at 13444, paragraph 2.

26. Triazole: the Keystone in Glycosylated Molecular Architectures Constructed by a Click Reaction

" The high affinity of the azide[12] for the alkyne group(chemoselectivity) and, by contrast, the inertness of both functionalities toward the majority of functional groups connected to the core of a variety of biomolecules (bioorthogonality), as well as the stability of the triazole ring toward chemical and enzymatic degradation,[13] are all features that concur to make this click reaction particularly suitable for covalently linking bioactive molecular entities, an operation in which mild and neutral conditions are a prerequisite." Dondoni, A. *Chem. Asian. J.* (2007) **2** 700- 708 at 702, paragraph 2.

27. The Rise of Azide-Alkyne 1,3-Dipolar 'Click' Cycloaddition and its Application to Polymer Science and Surface Modification

"As the cycloaddition is a coupling reaction, essentially all variations have been reported such as end group reaction, side chain or orthogonal reaction, surface coupling, and step growth polymerization." Evans, R.A. *Aust. J. Chem.* (2007) **60** 384-395 at 387, paragraph 2

28. <u>Azide-Modified Graphitic Surfaces for Covalent Attachment of Alkyne-Terminated Molecules by 'Click' Chemistry</u>

"In conclusion, a method to couple functional molecules to graphitic carbon surfaces through click chemistry has been developed. The wide variety of functional groups that are orthogonal to the click reaction and the stability of the 1,2,3-triazole promise a wide range of applications for these surfaces." Devadoss, A. and Chidsey, C.E.D. *J. Am. Chem. Soc.* (2007) **129** 5370-5371 at 5371, paragraph 3.

29. <u>Conceptual, Qualitative, and Quantitative Theories of 1,3-Dipolar and Diels-Alder Cycloadditions Used in Synthesis</u>

"The "click chemistry" methodology developed by the Sharpless group has been applied in a wide range of biological and materials chemistry contexts in the relatively short time since being introduced.[128] Arguably, the most appealing aspect of this strategy is the involvement of complementary substrates that are orthogonal to other functional groups and which participate in highly exothermic reactions." Ess, D.H. et al., *Adv. Synth. Catal.* (2006) **348** 2337-2361 at 2354, paragraph 5.

30. <u>A Simple Procedure for Connecting Two Carbohydrate Moieties by Click Chemistry Techniques</u>

"Interest in this reaction became clear after the recent discovery of the advantages of CuI as catalyst, reported independently by Sharpless and Meldal groups. This reaction in part owes its usefulness to its high compatibility with functional groups (alcohols, carboxylic acids, amines) in different solvent systems, including water." Gouin, S.G., et al., *Eur. J. Org. Chem.* (2007) 1160-1167 at 1160, paragraph 3.

forfeiture of a vessel without notice and hearing until after seizure. Police had found marijuana on the owner's yacht while in the possession of a lessee who chartered the boat. The statute did not require notice prior to seizure and did not exempt property of an owner who was neither involved in nor aware of the act of his lessee which resulted in the forfeiture. In dicta, the Court said,

> ... it would be difficult to reject the constitutional claim of an owner whose property subjected to forfeiture had been taken from him without his privity or consent. Similarly, the same might be said of an owner who proved not only that he was uninvolved in and unaware of the wrongful activity, but also that he had done all that reasonably could be expected to prevent the proscribed use of his property; for in such circumstance, it would be difficult to conclude that forfeiture served legitimate purposes and was not unduly oppressive.

Id. 416 U.S. at 689-90, 94 S.Ct. at 2094-95 (citations omitted). Wal-Mart also relies on a Second Circuit decision to support its assertion that there is a constitutional "innocent owner" defense to customs violations. *United States v. One Tintoretto Painting,* 691 F.2d 603, 607-08 (2d Cir. 1982) (owner of the painting attained ownership before the illegal importation into the U.S. and did all that reasonably could be expected to prevent the proscribed use of his property before letting another take it on commission to sell in the U.S.). However, as the Sixth Circuit recognized, both the *Calero-Toledo* and *Tintoretto Painting* cases involved situations where the owner of the property subject to forfeiture attained ownership rights prior to the illegal use of the property. *United States v. One 1984 Mercedes Benz Model No. 380 SE,* 836 F.2d 268, 270 (6th Cir. 1988) (stating that in those cases where the innocent owner defense was successful, the owner's rights to the property did not flow from the illegal activity itself).

[4] By contrast, Wal-Mart's ownership of the watches arises only after the unauthorized importation. Wal-Mart seems to suggest that we apply an "innocent purchaser for value" defense here. But, the Sixth Circuit has held, in an illegal importation case that *Calero-Toledo* does not create this defense. *See, One 1984 Mercedes Benz Model No. 380 SE,* 836 F.2d at 270 (no innocent purchaser defense for one who purchased vehicle from importer who falsely claimed that the vehicle was for personal use only and paid no duty because allowing defense would seriously undermine the enforcement of the customs laws). The Sixth Circuit decision was followed by the Eighth Circuit in *United States*

v. One Cessna Model 210L Aircraft, 890 F.2d 77, 82 (8th Cir. 1989) (alternatively holding that appellant's ownership of plane flowed from another's illegal drug trafficking and ordering forfeiture). Since Wal-Mart purchased these watches after their unauthorized importation, we hold that no innocent purchaser or owner defense is available to Wal-Mart. It would seem to render useless the current system of public recordation if purchasers of imported items could ignore the listings and obtain good title by simply asking their sellers, as Wal-Mart did, whether the imports were authorized.

AFFIRMED.

Court of Appeals, Federal Circuit

Miles Laboratories Inc. v. Shandon Inc.

Nos. 92-1358, -1387

Decided June 14, 1993

JUDICIAL PRACTICE AND PROCEDURE

1. Procedure — Judicial review — Standard of review — Patents (§410.4607.09)

Appellate review of federal district court's findings of fact in patent infringement action is under "clearly erroneous" standard, and district court's legal conclusions will be accepted unless incorrect as matter of law; appellate court will not reverse simply because it would have decided case differently.

PATENTS

2. Patentability/Validity — Specification — In general (§115.1101)

"Distinctly claiming" requirement of 35 USC 112, paragraph 2, means that claims must have clear and definite meaning when construed in light of complete patent documents; test for definiteness is whether one skilled in art would understand bounds of claim when read in light of specification, and degree of precision necessary for adequate claims depends upon nature of subject matter.

3. Infringement — Construction of claims (§120.03)

JUDICIAL PRACTICE AND PROCEDURE

Procedure — Judicial review — Standard of review — Patents (§410.4607.09)

Claim interpretation, as first step in two-part determination of whether infringement

occurred, proceeds as question of law, but if trial court resolves factual disputes underlying meaning of claim terms, then those findings are reviewed on appeal under "clearly erroneous" standard.

PATENTS

4. Infringement — Construction of claims (§120.03)

Infringement — Literal infringement (§120.05)

Patent construction — Claims — Defining terms (§125.1305)

Federal district court, in considering claims for light microscopy processing apparatus, properly construed term "cabinet" to mean single enclosure for various parts of apparatus, but court clearly erred in finding that accused device, which consists of three cabinets, literally infringes single cabinet limitation of patent in suit.

5. Infringement — Doctrine of equivalents — In general (§120.0701)

Federal district court did not err in determining that accused device for light microscopy processing apparatus infringed under doctrine of equivalents, even though defendant contends that intended result of patent in suit is unification of various components in single cabinet, while accused device achieves safety and operational advantages by separating components, since infringement under doctrine of equivalents does not vanish merely because accused device performs functions in addition to those performed by claimed device, since accused device, regardless of its separation of components into modules, still achieves substantially same result as patent in suit, and since allowing defendant to escape infringement simply through its use of separate cabinets would cause injustice that doctrine of equivalents is designed to prevent.

6. Patentability/Validity — Obviousness — Secondary considerations generally (§115.0907)

Objective indicia of non-obviousness weigh in favor of finding of non-obviousness, although lack of such evidence does not weigh in favor of obviousness.

7. Patentability/Validity — Obviousness — Relevant prior art — Particular inventions (§115.0903.03)

Federal district court did not err in finding claims for light microscopy processing

method to be invalid for obviousness, in view of evidence demonstrating that differences between prior art and patent in suit were minor and achievable by simple modification, and demonstrating that prior art references collectively suggest engineering necessary to achieve these modifications, and in view of plaintiff's failure to show any objective indicia of non-obviousness.

Particular patents — General and mechanical — Light microscopy

Re. 29,073 (3,892,197), Kinney and Pickett, light microscopy processing apparatus, holding of validity and infringement affirmed.

Particular patents — Chemical — Light microscopy

4,001,460, Kinney and Pickett, light microscopy processing method, holding of invalidity affirmed.

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Appeal from the U.S. District Court for the Western District of Pennsylvania, Diamond, J.

Action by Miles Laboratories Inc. and Triangle Biomedical Equipment Inc. against Shandon Inc. and Shandon Southern Products Limited, for patent infringement. From federal district court decision holding one patent in suit invalid for obviousness, and holding other patent valid and affirmed, parties cross-appeal. Affirmed.

Arnold Sprung, of Sprung Horn Kramer & Woods (Nathaniel D. Kramer, with him on brief), Tarrytown, N.Y., for plaintiffs.

Robert D. Yeager, of Kirkpatrick & Lockhart (Christine R. Ethridge and Melvin C. Snyder, III, with him on brief), Pittsburgh, Pa., for defendants.

Before Smith, senior circuit judge, and Plager and Rader, circuit judges.

Rader, J.

Miles Laboratories, Inc. and Triangle Biomedical Equipment, Inc. sued Shandon Inc. and Shandon Southern Products Limited, for infringement of U.S. Patent Reissue No. 29,073, entitled "Light Microscopy Processing Apparatus" ('073),* and U.S. Patent No. 4,001,460, entitled "Light Microscopy

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* U.S. Patent Reissue No. 29,073 issued on December 14, 1976 as a reissue of U.S. Patent No. 3,892,197, which issued on July 1, 1975).

Processing Method" ('460). The United States District Court for the Western District of Pennsylvania held claims 1, 2, and 4-7 of the '460 patent invalid for obviousness, sustained the validity of the '073 patent, and found infringement of both patents. *Miles Lab., Inc. v. Shandon, Inc.*, No. 86-2404 (W.D. Pa. Mar. 11, 1992) (*Miles I*); *Miles Lab., Inc. v. Shandon, Inc.*, No. 86-2404 (W.D. Pa. Apr. 14, 1992) (*Miles II*). Because the record adequately supports the district court's decision, this court affirms.

BACKGROUND

Tissue processing is the treatment of tissue specimens to facilitate viewing them under a microscope. The process exposes the issue specimens to a series of chemical solutions (reagents) in sequence. The '460 patent claims a method and the '073 patent an apparatus for tissue processing. Except for the claims, the two patents have identical specifications.

Under the method accomplished by the apparatus, a central processing chamber confines the tissue specimens under a sealed cover where they remain fixed during treatment with various fluids and paraffin. Once embedded in paraffin, the specimens can be sliced into very thin sections for microscopic viewing. The treatment takes place when a vacuum draws the fluids and paraffin into the central chamber. After proper exposure, pressure in the central chamber expels the fluids back to their storage containers. Thus, the entire processing occurs without tampering with the tissue specimens.

In 1986, Miles sued Shandon for infringement of both patents. The district court held a bench trial in 1988. The district court determined that the doctrine of laches did not bar this action and that claim 1 of the "460 patent was invalid under 35 U.S.C. § 103. *Miles I*, slip op. at 30. The district court also upheld the validity of the '073 patent and found infringement of both patents. *Id.*

Later, the district court clarified its earlier decision and added the '460 patent's dependent claims 2 and 4-7 to its obviousness ruling. *Miles II*, slip op. at 1. In addition, the district court enjoined Shandon from further infringement of the '073 patent. *Id.* Shandon appeals the validity determination on the '073 patent and the infringement rulings. Miles cross-appeals the invalidity determination on the '460 patent.

DISCUSSION
Standard of Review

This court reviews the district court's fact finding under the "clearly erroneous" standard of Rule 52(a):

Findings of fact, whether based on oral or documentary evidence, shall not be set aside unless clearly erroneous, and due regard shall be given to the opportunity of the trial court to judge of the credibility of the witnesses.

Fed. R. Civ. P. 52(a) (1988); *see Heisig v. United States*, 719 F.2d 1153, 1158 (Fed. Cir. 1983). This court accepts the legal conclusions of the district court unless incorrect as a matter of law. *Id.*

[1] This court does not review *de novo* proceedings of the district court. *Medtronic, Inc. v. Daig Corp.*, 789 F.2d 903, 904, 229 USPQ 664, 666 (Fed. Cir.), *cert. denied*, 479 U.S. 931 (1986). To win reversal, a party must show that the district court committed reversible legal error or relied upon factual findings which were clearly erroneous in light of the trial record. *Id.* at 904-05. In addition, the "clearly erroneous" standard does not entitle this court to reverse the district court's finding simply because it would have decided the case differently. *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1375, 231 USPQ 81, 87 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). Where the factfinder's account of the evidence is plausible in light of the entire record or where it chooses one of two permissible views of the evidence, it has committed no clear error. *Id.*

The '073 Patent

On the last day of trial, Shandon moved to introduce an infringement defense that the '073 patent was invalid for indefiniteness under 35 U.S.C. § 112, ¶ 2 (1988). The district court, however, upheld the validity of the '073 patent. On appeal, Shandon alleges the claims of the '073 patent omit the requirement for "vented" solution containers and therefore do not distinctly claim the disclosed invention.

Validity

[2] Shandon challenged the claims of the '073 patent as indefinite under § 112, ¶ 2. Compliance with § 112, ¶ 2 is a question of law. *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576, 1 USPQ2d 1081, 1088 (Fed. Cir. 1986). Section 112, paragraph 2, states:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

35 U.S C. §112, ¶ 2. The "distinctly claiming" requirement means that the claims must have a clear and definite meaning when construed in the light of the complete patent document. *Standard Oil Co. v. American Cyanamid Co.,* 774 F.2d 448, 452, 227 USPQ 293, 296 (Fed. Cir. 1985). Section 112 thus ensures definiteness of claim language. *See In re Zletz,* 319, 322, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989).

The test for definiteness is whether one skilled in the art would understand the bounds of the claim when read in light of the specification. *Orthokinetics,* 806 F.2d at 1576. If the claims read in light of the specification reasonably apprise those skilled in the art of the scope of the invention, § 112 demands no more. *Hybritech,* 802 F.2d at 1385. The degree of precision necessary for adequate claims is a function of the nature of the subject matter. *Id.*

At trial, a Miles expert, Mr. Kocsis, stated:

Q Now, reading these claims [of the '073 patent], which we have just discussed, did you see any mention in any of these claims of vented containers or reagent bottles, or anything like that?
A No, I did not.

. . . .

Q Now, that single machine, as described in the '460 and '073 patents, requires that a vent to atmosphere be present in each solution container in order for the machine to transfer solutions from a solution container to a processing chamber and back, is that correct?
A That's correct.

Relying on these isolated statements, Shandon contends that the claims do not specify vented solution containers. Without vented containers, Shandon contends, the claims do not describe a workable invention. Without vents, Shandon asserts, the invention cannot change pressure to draw fluids into and out of the central treatment chamber.

Shandon's argument is irrelevant to definiteness under § 112, ¶ 2. The invention's operability may say nothing about a skilled artisan's understanding of the bonds of the claim. Shandon's argument is possibly relevant, however, to the enablement requirement of § 112, ¶ 1, or to utility under § 101.

Construed as a challenge to utility or enablement, Shandon's argument nevertheless fails. Mr. Kocsis testified that the claimed tissue processors would operate with or without vents in the solution containers. Without vents, collapsible solution containers could permit the transfer of fluids by pressure changes. The district court correctly concluded that "the record shows that even unvented containers would be operative." *Miles II,* slip op. at 4. Thus Shandon did not show a lack of utility, even if the claims cover only unvented containers.

The trial court also determined that the claims, read in light of the specification, covered both unvented containers and vented containers. In fact, the preferred embodiment described in the specification discloses "vented" solution containers:

Referring again to FIG 3, the previously referred to solution containers 15 (with operating numbers 1 through 10) have respective caps 55 for refilling the containers. Suitable air vents 56, indicated by dashed lines, are provided in each cap 55, but are preferably kept extremely small so as to limit any admission of moisture.

Col. 6, lines 3-9. Therefore, the claims read in light of the specification reasonably apprise those skilled in the art of the claimed invention. Moreover, the record shows that the patent disclosed adequate information to enable a skilled artisan to make and use the claimed invention. *Northern Telecom, Inc. v. Datapoint Corp.,* 908 F.2d 931, 941, 15 USPQ2d 1329 (Fed. Cir.), *cert. denied,* 498 U.S. 920 (1990).

Appellant incorrectly characterized its validity challenge as a claim definiteness issue — a characterization which the district court followed, at least in name. Nonetheless, the district court made proper findings and correctly concluded that appellant did not rebut the presumed validity of the claims.

Infringement

The district court determined that the accused devices, known as the HYPER-CENTER and the HYPERCENTER 2, infringed the '073 patent literally, or in the alternative, under the doctrine of equivalents. *Miles I,* slip op. at 28-30. On appeal, Shandon argues that the district court misconstrued the "cabinet" limitation of the claims.

[3] This court reviews a trial court's infringement findings under the "clearly erroneous" standard. *Charles Greiner & Co. v. Mari-Med Mfg., Inc.,* 962 F.2d 1031, 1034, 22 USPQ2d 1526, 1528 (Fed. Cir. 1992); *Insta-Foam Prods., Inc. v. Universal Foam Sys., Inc.,* 906 F.2d 698, 702, 15 USPQ2d 1295, 1297 (Fed. Cir. 1990). Claim interpre-

tation is the first step in the two-part infringement determination. *Greiner*, 962 F.2d at 1034. Claim interpretation proceeds as a question of law. *Id.* When a trial court, however, resolves factual disputes underlying the meaning of claim terms, this court reviews these findings under the clearly erroneous standard. *Id.* In interpreting disputed claim terms, the trial court considers the specification and the prosecution history. *Id.* After interpreting the claim, the final step of the infringement analysis determines whether the accused device is within the scope of the claim. *Id.* To infringe, an accused device must embody exactly each claim limitation or its equivalent. *Id.*

The district court determined that the HYPERCENTERs contained every limitation set forth in claim 1 of the '073 patent. *Miles I,* slip op. at 28. In reaching this conclusion, the district court construed the cabinet limitation of claim 1 to define an enclosure for the various elements of the processing apparatus. *Id.* The court also determined that the HYPERCENTERs consisted of three modules: a module which housed the operating controls, a module which housed the reagent storage bottles, and a module which contained the central processing chamber and the paraffin baths. The district court concluded that the separate modules of the HYPERCENTER collectively formed a cabinet. *Id.*

[4] The district court properly construed the term "cabinet" to mean a single enclosure for the various parts of the apparatus. The claims, specification, and drawings disclose a single cabinet enclosing the tissue processing apparatus. The embodiment illustrated in the patent specification disclosed a single cabinet comprised of a number of sections, including numerous reagent bottles, a processing chamber, paraffin containers, and a control module. Moreover, Webster's defines "cabinet" as "1 a case or cupboard with drawers or shelves for holding or storing things . . . 2 a boxlike enclosure." *Webster's New World Dictionary,* 193 (3d col. ed. 1988).

The HYPERCENTERs, however, consist of three modules as opposed to one. "Module" is defined as "any of a set of units, as cabinets, designed to be arranged or joined in a variety of ways." *Webster's* at 872. Because three does not equal one, the district court clearly erred in finding that the HYPERCENTERs (consisting of three cabinets) literally infringed the single cabinet limitation of the '073 patent.

[5] This court, however, concludes that the district court did not err in determining that

the HYPERCENTERs infringed the '073 patent under the doctrine of equivalents. Infringement under the doctrine of equivalents requires a showing that the accused device performs substantially the same function, in substantially the same way, to achieve substantially the same result as the claimed device. *Malta v. Schulmerich Carillons, Inc.,* 952 F.2d 1320, 1325, 21 USPQ2d 1161, 1165 (Fed. Cir. 1991), *cert. denied,* 112 S. Ct. 2942 (1992) (citing *Graver Tank & Mfg. Co. v. Linde Air Prods. Co.,* 339 U.S. 605, 608 [85 USPQ 328] (1950)).

The doctrine of equivalents prevents the pirating of the patentee's invention in the absence of literal infringement when liability is nevertheless warranted. *Hormone Research Found., Inc. v. Genetech, Inc.,* 904 F.2d 1558, 1564, 15 USPQ2d 1039, 1044 (Fed. Cir. 1990). The doctrine of equivalents thus prevents the risk of injustice that may result from a limited focus on words alone. *Laitram Corp. v. Cambridge Wire Cloth Co.,* 863 F.2d 855, 856-57, 9 USPQ2d 1289, 1291 (Fed. Cir. 1988), *cert. denied,* 490 U.S. 1068 (1989).

Shandon argues that the district court did not determine that the HYPERCENTERs achieved "substantially the same result" as the '073 patent. Shandon contends that the intended result of the '073 patent is unification of the various components. Shandon alleges that HYPERCENTERs achieve safety and operational advantages by separating the components.

The '073 patent achieves an enclosed tissue processing system. The district court stated:

> The '073 patent discloses an *apparatus* for fixing and processing the tissue specimens. It is an improvement over the prior art because it represents the first completely automatic system for allowing light microscopy tissue to be processed under a completely automatic sequence in an entirely closed system and without requiring substantial movement of the specimens.

Miles I, slip op. at 3-4 (citation omitted). This result does not change merely because Shandon separated certain components of the system into discrete modules.

In addition, the '073 patent does not specify that the cabinet contains all components of the invention. Rather claim 1 specifies an "air pump means . . . mounted proximate said cabinet." The '073 patent, col. 11, lines 17-19. Claim 1 also claims "electrical control means . . . mounted proximate said chamber." *Id.* col. 12, lines 1-3. Therefore, al-

though claim 1 may have a cabinet limitation, not all components of the tissue processor must be within the cabinet. Indeed, the specification states that "the controls could be mounted in a separate cabinet." *Id.* col. 10, lines 34-35.

The limitations and functions of the invention in the claims, not the elements or functions of the accused device, establish the reference point for the doctrine of equivalents. *Insta-Foam*, 906 F.2d at 702. Infringement under the doctrine does not vanish merely because the accused device performs functions in addition to those performed by the claimed device. *Id.* Regardless of separation into modules, Shandon's system is still a "completely automatic system for allowing light microscopy tissue to be processed under a completely automatic sequence in an entirely closed system and without requiring substantial movement of the specimens." *See Miles I,* slip op. at 3-4. Thus, the HYPERCENTERs achieved substantially the same result as the '073 patent.

To allow Shandon to escape infringement simply because it used separate cabinets, as opposed to a single cabinet, is the exact type of injustice the doctrine of equivalents prevents. *See Laitram Corp.,* 863 F.2d at 856-57. This court discerns no clear error in the district court's finding of infringement under the doctrine of equivalents.

The '460 Patent

The district court held claim 1 of the '460 patent invalid for obviousness under 35 U.S.C. § 103 (1988). *Miles I,* slip op. at 16-17. The district court later held the dependent claims of the '460 patent (claims 2, 4-7) invalid by virtue of claim 1's invalidity. *Miles II,* slip op. at 2.

35 U.S.C. § 103 — Obviousness

The ultimate legal conclusion of obviousness is a question of law. *Specialty Composites v. Cabot Corp.,* 845 F.2d 981, 989, 6 USPQ2d 1601, 1606 (Fed. Cir. 1988). The analysis of obviousness, however, rests on several factual inquiries: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims; (3) the level of ordinary skill in the art at the time of invention; and (4) objective evidence of nonobviousness. *Id.* (citing *Graham v. John Deere Co.,* 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966)). This court reviews these factual underpinnings for the legal conclusion of obviousness under the "clearly erroneous" standard. *Specialty Composites,* 845

F.2d at 989. The facts stated herein are based on district court findings not found to be clearly erroneous or otherwise not in dispute.

The prior art in this instance included U.S. Patent No. 3,526,203 (the '203 patent), U.S. Patent No. 3,227,130 (the Weiskopf patent), and the Lipshaw Manufacturing Corporation's "Fluid X Changer." The '203 patent covers an electron microscopy tissue processor. Electron microscopy differs from light microscopy in that the former requires only very small tissue specimens. With small tissue specimens, electron microscopy does not need to reuse processing reagents. Nonetheless, the specification of the '203 patent provides: "it will be apparent that the processor of the invention may be used for processing the larger sized tissue particles which are intended for light microscopy examination." *Miles I,* slip op. at 10 (quoting U.S. Patent No. 3,526,203, col. 8, lines 5-8). The claims of the '203 patent disclose the vacuum component of the '460 patent. Furthermore, the '203 patent suggests a solution to the problem resolved by claim 1 of the '460 patent, namely, a means of reusing a solution by returning unused quantities to the storage container with pressure.

The specification of the '203 patent provides:

> In this regard it should be noted that the practice in electron microscopy work is not to reuse the solutions and in the system of the invention only fresh solution is transferred through the lines and valves connecting the containers with the processing chamber. If the particular solutions are required to be pumped back to the containers after use appropriate pumping and switching controls would have to be provided.

U.S. Patent NO. 3,526,203, col. 8, lines 12-19. Although electron microscopy does not reuse solutions, the '203 patent suggests to a skilled artisan the reuse of solutions by pumping them back to their storage containers.

The "Fluid X Changer" (a device used for sustaining slides bearing tissue specimens) also suggests transfer of solutions by pressure. Moreover, the Weiskopf patent discloses a tissue processor which transfers solutions by pressure controls. Thus, the prior art of histological equipment taught the flow of liquids in tissue processing apparatuses from one location to another with vacuum-pressure.

The differences between the prior art and claim 1 of the '460 patent were minor and achievable by simple modification. Moreover, the prior art references collectively sug-

gest the engineering necessary to achieve these modifications. Simply put, the '203 patent discloses a tissue processor which does not reuse fluids but instead discharges them into a waste tank after processing. By running a line from the processing chamber back to the fluid storage containers (rather than to the waste tank), the '203 patent would anticipate the '460 patent.

[6] The level of ordinary skill in the art suggests as well a thorough knowledge of the principles of fluid transfer using pressure-vacuum pumps, valves, and conduits at the time of the '460 patent's development. Finally, Miles did not show objective indicia of non-obviousness. Such evidence, if present, would weigh in favor of non-obviousness, although the lack of such evidence does not weigh in favor of obviousness. *See, e.g., Custom Accessories, Inc. v. Jeffrey-Allan Indus., Inc.*, 807 F.2d 955, 1 USPQ2d 1196, 1199 (Fed. Cir. 1986). Miles presented no evidence, for instance, that its device represented a substantial share of any definable market. Miles also did not offer evidence on factors such as long-felt need or teaching away in the prior art.

[7] In sum, the district court concluded:
On the basis of the *Graham* test, therefore, we conclude that claim 1 of the '460 patent is invalid under 35 U.S.C. § 103 because the subject matter of claim 1 as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which the subject matter pertains.

Miles I, slip op. at 16-17. This court finds no error (and certainly no clear error) with the district court's obviousness findings and conclusion. Therefore this court affirms the district court's determination of invalidity of claim 1 of the '460 patent.

In its later opinion, the district court clarified its earlier decision and also held dependent claims (2 and 4-7) of the '460 patent invalid. *Miles II,* slip op. at 1-2. Section 282 requires an independent analysis of the validity of each claim. 35 U.S.C. § 282 (1988); *Ortho Pharmaceutical Corp. v. Smith,* 959 F.2d 936, 942, 22 USPQ2d 119, 1124 (Fed. Cir. 1992). A party challenging the validity of a claim, absent a pretrial agreement or stipulation, must submit evidence supporting a conclusion of invalidity for each contested claim. *Id.* Where the parties stipulate to "representative" claims, however, a validity resolution for the representative claims applies to the other claims as well. *See Panduit Corp. v. Dennison Mfg. Co.,* 836 F.2d 1329, 1330-31, 5 USPQ2d 1266, 1267-68 (Fed. Cir. 1987).

In an April 1988 pretrial "Stipulation of Agreed Fact, Law of the Case and Questions of Law," the parties agreed:
The '460 patent contains seven claims. Claim 1 is the only independent claim. Claims 2 through 7 depend directly or indirectly from claim 1. Consequently, claim 1 is the broadest claim and can be considered to be representative of the claims in this patent.

Miles II, slip op. at 2 n.1. This stipulation of the parties made claim 1 a representative for the other claims in the patent. Thus, the parties, their counsel, and the trial court understood that the result the court reached for claim 1 would bind all other claims. Therefore, this court affirms the district court's invalidation of the dependent claims of the '460 patent.

The district court also determined that the accused device infringed the '460 patent. Because it affirms the district court's invalidity findings, this court need not reach the district court's infringement determination. *See Dana Corp. v. IPC Ltd. Partnership,* 860 F.2d 415, 417, 8 USPQ2d 1692, 1694 (Fed. Cir. 1988), *cert. denied,* 490 U.S. 1067 (1989).

CONCLUSION

For the above stated reasons, this court affirms the district court's findings of infringement of the '073 patent and the upholding of its validity. This court also affirms the district court's holding that claims 1, 2, and 4-7 of the '460 patent are invalid due to obviousness.

COSTS

Each party shall bear its own costs for this appeal.

AFFIRMED

Court of Appeals, Federal Circuit

McElmurry v. Arkansas Power & Light Co.

No. 92-1246

Decided June 16, 1993

JUDICIAL PRACTICE AND PROCEDURE

1. Procedure — Summary judgment — Patents (§410.3303)

Procedure — Judicial review — Standard of review — Patents (§410.4607.09)

Appeals court, in reviewing federal district court's grant of summary judgment in

clauses can have no meaning under the third paragraph of § 112," citing the following statement in In re Jones, 54 CCPA 1218, 373 F.2d 1007, 153 USPQ 77 (1967): "Appellant cannot positively claim what he has not disclosed and he has disclosed no means." This argument appears to raise the issue as to whether the language of the *third* paragraph of § 112 may be viewed as requiring the disclosure of at least one specific means in order to support a means clause in a claim.

We decline to speculate as to what specific grounds the board intended in affirming this rejection or regarding the possible positions the parties might take on the various issues we have indicated could be present here. Accordingly, we remand this case for clarification of this rejection and further consideration of all issues regarding these claims. Cf., In re Frilette, 57 CCPA 991, 423 F.2d 1397, 165 USPQ 259 (1970).

Summary

The decision of the Board of Appeals with regard to claims 1, 4, 5, 7, 8, 12, 15 and 16 is *affirmed.* The case, insofar as the rejection of the remaining claims is concerned, is *remanded.*

Court of Customs and Patent Appeals

In re MOORE AND JANOSKI

No. 8428   Decided Apr. 1, 1971

PATENTS

1. Claims — Indefinite — In general (§20.551)

   Construction of specification and claims — By prior art (§22.20)

   Construction of specification and claims — By specification and drawings — In general (§22.251)

   Definiteness of language employed in claims must be analyzed — not in a vacuum, but always in light of teachings of prior art and of the particular application disclosure as it would be interpreted by one possessing ordinary level of skill in pertinent art; claims may appear indefinite when read in a vacuum, but may be definite upon reading of specification or prior art teachings; an otherwise definite claim in a vacuum may be un-

certain when read upon specification and prior art.

2. Claims — Specification must support (§20.85)

   Specification — Sufficiency of disclosure (§62.7)

   Once having determined that subject matter defined by claims is particular and definite, analysis turns to first paragraph of 35 U.S.C. 112 to determine whether scope of protection sought is supported by specification disclosure; inquiries include determining whether subject matter defined in claims is described in specification, whether specification disclosure as a whole is such as to enable one skilled in the art to make and use claimed invention, and whether best mode contemplated by inventor of carrying out that invention is set forth.

   Particular patents—Alkyladamantanes
   Moore and Janoski, Highly Fluorinated Alkyladamantanes, claims 1 to 7 of application allowed.

Appeal from Board of Appeals of the Patent Office.

Application for patent of Robert E. Moore and Edward J. Janoski, Serial No. 440,614, filed Mar. 17, 1965; Patent Office Group 120. From decision rejecting claims 1 to 7, applicants appeal. Reversed.

BARRY A. BISSON, Wilmington, Del., for appellants.
S. WM. COCHRAN (JOSEPH F. NAKAMURA of counsel) for Commissioner of Patents.

Before RICH, ALMOND, BALDWIN, and LANE, Associate Judges, and DURFEE, Judge, United States Court of Claims, sitting by designation.

BALDWIN, Judge.

The sole issue in this appeal is whether the Patent Office Board of Appeals was correct in affirming the rejection of claims 1-7 in appellants' application[1] as failing to comply with the requirements of 35 U.S.C. 112. Five other claims were allowed.

The Invention

The subject matter of the appealed claims involves products which are obtained when hydrocarbon compounds of the following nature are fluorinated:

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[1] Serial No. 440,614, filed March 17, 1965, for "Highly Fluorinated Alkyladamantanes."
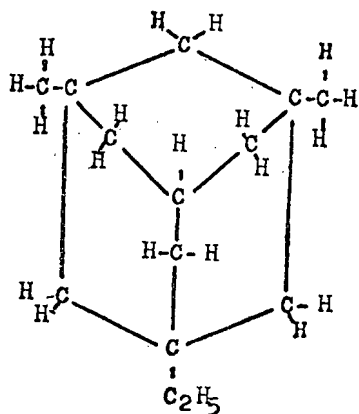
The two-dimensional drawing shown above is supposed to represent a highly complex, three-dimensional compound referred to as an alkyl adamantane. According to appellants' specification, the carbon atoms of the basic adamantane nucleus are "arranged in a completely symmetrical, strainless manner such that four of the carbon atoms occupy bridgehead positions." This arrangement, and the fact that dehydrogenation cannot occur because of the bridgehead carbon atoms, produces a "highly stable hydrocarbon." The specification goes on to teach that when "these alkyladamantanes are subjected to fluorinating conditions effective to result in a degree of fluorination which is at least 75% of that corresponding to perfluorination," there results a highly fluorinated product with some very desirable properties.

The rejected claims fall into two groups. Claims 1 and 2 make up the first group, of which claim 1 is illustrative:

> 1. As a composition of matter, a mixture of highly fluorinated alkyladamantanes prepared by fluorinating an alkyladamantane
>
> selected from the group consisting of 1, 3-dimethyladamantane, 1, 3, 5-trimethyladamantane, 1-ethyladamantane, 1-ethyl-3-methyladamantane and 1-ethyl-3, 5-dimethyladamantane,
>
> and in which the degree of fluorination is at least 75% of that corresponding to perfluorination of said alkyladamantane.

Claims 3-7 are directed to compositions formed from specific alkyladamantanes. Claim 3 is illustrative:

> 3. As a composition of matter, highly fluorinated 1-ethyladamantane containing at least 15 fluorine atoms per molecule.

The allowed claims are directed to per-

fluorinated compositions, *i.e.*, wherein all available hydrogens are replaced by fluorine atoms. No references have been relied on.

The Rejection

It appears from the record that while appellants' claims were consistently rejected under 35 U.S.C. 112, the specific grounds for so rejecting were not fully articulated until the examiner's Answer to appellants' brief before the Board of Appeals. With respect to claims 1 and 2, the examiner there took the position that the recitation therein of "a mixture of highly fluorinated alkyladamantanes prepared by fluorinating an alkyladamantane" rendered those claims product-by-process claims. He went on to hold that, as such, the claims were indefinite since in the absence of a recitation of specific process limitations "it is not clear which products are produced" or "whether a given fluorination process will preferentially substitute the ring, chain or bridgehead carbon atoms".

As to claims 3-7, which employ the language "highly fluorinated" to modify the particular alkyladamantane recited, the examiner stated first that he was unsure "whether a regular generic product or product-by-process claim is intended." He then went on to indicate that if these claims were to be regarded as product-by-process claims he would hold them indefinite on the same grounds applied to claims 1 and 2. These grounds he later summarized as "there are insufficient process limitations to define the product produced by said process." If, on the other hand, the claims were intended to be "regular generic product" claims, the following alternative ground of rejection was set forth:

> there is no evidence that any particular product within the scope of the claims can be prepared at will nor is there any disclosure of a single species. Thus there is no support for a claim generic to all conceivable species when only certain mixtures can be prepared.

Again, in response to certain of appellants' arguments, the examiner later expanded on this reasoning as follows:

> in order for the disclosure to properly support these claims *it must be evident that all individual isomers covered can be prepared.* The present record does not make this evident. Even assuming that random fluorination occurs, the genus is readable on compounds which are not randomly fluorinated, e.g. those which have no fluorine on the alkyl groups, with all of the fluorine on the rings and bridge-

head. Thus, it is clear that these unsymmetrically fluorinated products cannot be prepared by random fluorination, yet they are encompassed by the claims. [Emphasis quoted.]

It seems indisputable that the examiner's reasoning in support of the rejection before us, as finally articulated, set forth two separate grounds for rejection. The first was that *all* of the claims, when viewed as product-by-process claims, are lacking in definiteness as required by the *second* paragraph of 35 U.S.C. 112. The second, applicable only to claims 3-7 and clearly alternative rather than cumulative to the first ground, was that, if these claims were viewed as being drawn to generic product coverage, they would not be supported by an adequate enabling disclosure as required by the *first* paragraph of § 112.

The Board of Appeals found "no reversible error in the rejection of the claims under 35 U.S.C. 112." However, for whatever reasons they saw fit, the board chose only to treat the question of the claims' definiteness, stating:

> We view the claims before us as drawn to incomplete and therefore indefinite product-by-process claims in the language "highly fluorinated alkyladamantanes prepared by * * *" (claims 1-2) and "highly fluorinated" (claims 3-7). It is our view that the omission from the claims of process parameters, requisite to yield the desired fluorinated alkyladamantanes, renders the claims indefinite. It is too well-known in the art to belabor, that fluorination reactions broadly would yield not only the desired fluorinated alkyladamantanes but also as presented, undesired fluorinated degradation products of the alkyladamantanes.
>
> With respect to Claims 3 through 7, we find the additional infirmity that while the language of each of these claims seems to be directed to a single compound, Claims 3 through 7 cover, in fact, any one or a mixture of more than one compound. If generic coverage was intended, appropriate definite generic claim language was available and no need is seen for resort to ambiguous claim terminology.

Opinion

The solicitor has not aided us in resolving the status of the issues on appeal, stating at one point in his brief that "an issue before the Court" is the correctness of the holdings by the examiner and the board that the claims are indefinite, and later on in his

argument that the claims "are broader than the disclosure *and thereby* fail to particularly point out and distinctly claim the invention." (Emphasis added). In any event we perceive, in the proceedings leading up to this appeal, a definite lack of sensitivity to the specific language of 35 U.S.C. 112 and a resultant confusion of a number of issues which, procedurally at least, should have been articulated and treated separately.

For the sake of completeness we will treat the claims on appeal as if they were rejected under both the first and second paragraphs of § 112. Any analysis in this regard should begin with the determination of whether the claims satisfy the requirements of the second paragraph. It may appear awkward at first to consider the two paragraphs in inverse order but it should be realized that when the first paragraph speaks of "the invention", it can only be referring to that invention which the applicant wishes to have protected by the patent grant, i.e., the *claimed* invention. For this reason the claims must be analyzed first in order to determine exactly what subject matter they encompass. The subject matter there set out must be presumed, in the absence of evidence to the contrary, to be that "which the applicant regards as his invention."

[1] This first inquiry therefore is merely to determine whether the claims do, in fact, set out and circumscribe a particular area with a reasonable degree of precision and particularity. It is here where the definiteness of the language employed must be analyzed—not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary level of skill in the pertinent art.[2]

[2] Once having determined that the subject matter defined by the claims is particular and definite, the analysis then turns to the first paragraph of section 112 to determine whether the scope of protection sought is supported and justified by the specification disclosure. This first paragraph analysis in itself contains several inquiries. Considering the language of the statute, it

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[2] It is important here to understand that under this analysis claims which on first reading — in a vacuum, if you will - appear indefinite may upon a reading of the specification disclosure or prior art teachings become quite definite. It may be less obvious that this rule also applies in the reverse, making an otherwise definite claim take on an unreasonable degree of uncertainty. See In re Cohn, 58 CCPA ___, ___ F.2d ___, 169 USPQ 95 (1971), In re Hammack, 57 CCPA 1225, 427 F.2d 1378, 166 USPQ 204 (1970).

should be evident that these inquiries include determining whether the subject matter defined in the claims is described in the specification, whether the specification disclosure as a whole is such as to enable one skilled in the art to make and use the claimed invention, and whether the best mode contemplated by the inventor of carrying out that invention is set forth.

Two of the first paragraph requirements indicated above, i.e., the "description of the invention" and the "best mode" requirements, are relatively simple to comply with and thus will ordinarily demand minimal concern on the part of the Patent Office. We recently have had occasion to discuss the first of these requirements. See In re Di-Leone, 58 CCPA __, 436 F.2d 1404, 168 USPQ 592 (1971), In re Ahlbrecht, 58 CCPA __, 435 F.2d 908, 168 USPQ 293 (1971), In re Robins, 57 CCPA 1321, 429 F.2d 452, 166 USPQ 552 (1970). As to the latter, see In re Gay, 50 CCPA 725, 309 F.2d 769, 135 USPQ 311 (1962). What is of maximum concern in any analysis of whether a particular claim is supported by the disclosure in an application is whether that disclosure contains sufficient teaching regarding the subject matter of the claims as to enable one skilled in the pertinent art to make *and* to use the claimed invention. These two requirements, "how to make" and "how to use" have sometimes been referred to in combination as the "enablement" requirement, but, in one form or another, have been the subject of extended discussion in this court of recent years. The relevant inquiry may be summed up as being whether the scope of enablement provided to one of ordinary skill in the art by the disclosure is such as to be commensurate with the scope of protection sought by the claims.

Applying now the analysis outlined above to the case before us, the position of the Board of Appeals regarding the definiteness of the claims before us can be justified only if it can be concluded that one of ordinary skill in this art, having appellants' disclosure and claims before him, would not be possessed of a reasonable degree of certainty as to the exact subject matter encompassed within the claims. We must conclude that the board's position cannot stand. We simply cannot understand why it is felt that process parameters are important here. Appellants clearly disclose that it is not the *type* of fluorination process which is important but the *fact* that the products are fluorinated and fluorinated to a specific minimum degree (spelled out by all the claims). When it was

questioned whether known fluorination processes would produce the same type of product, appellants submitted an affidavit by one Schneider, an expert in the field, stating the opinion that "alkyl adamantanes when fluorinated by any of such procedures to the extent of at least 75% of that corresponding to perfluorination will convert into substantially the same highly fluorinated products regardless of which fluorination procedure is used." In the absence of anything of substance to the contrary, we find this opinion conclusive. The board's further comment that fluorination processes also yield undesired degradation products would be pertinent to the issue of definiteness if it were true *and* if the word "fluorinated" had to be considered as a "product-by-process" limitation. The Schneider affidavit, however, not only rebuts the latter interpretation but also presents facts which appear to be inconsistent with the former assertion. In any event, since we have found that these claims are not product-by-process claims, such a contention, even if it were true would be irrelevant to the issue of what the claims define and whether they are definite since it is quite clear that appellants do not wish to claim these degradation products, if any, *but only* the highly fluorinated alkyl adamantanes which are not degraded.

Considering now any assertions that the claims are not supported by an adequate enabling disclosure and thus are unduly broad, we also find these to be not well taken. As appellants' disclosure makes clear and the Schneider affidavit confirms, when the recited alkyl adamantanes are fluorinated by known processes to a degree short of complete substitution of all hydrogen atoms, there occur mixtures of compounds randomly fluorinated to the specified degree. Of pertinence here is what we said in In re Steinhauer, 56 CCPA 1093, 410 F.2d 411, 161 USPQ 595, 599 (1969):

> What results from these procedures is not pure compounds but mixtures of compounds. That is what appellants want, it is what they get, and it is what they claim. Appellants are not claiming *any* specific compound. To demand of them that they shall turn their investigations in another direction and carry on an undetermined amount of further research to determine just *where all* the substituents are positioned on each ring in *each* compound in each mixture of each working example in their specification seems to us beyond the requirements of the law.

The decision of the Board of Appeals is *reversed*.

Court of Customs and Patent Appeals

In re BORREGARD

No. 8489   Decided Apr. 1, 1971

PATENTS

Particular patents—Transfer Sheet

Borregard, Dry Transfer Sheet and Method, claims 24 to 27 of application refused.

Appeal from Board of Appeals of the Patent Office.

Application for patent of Ernst Borregard, Serial No. 300,743, filed Aug. 8, 1963; Patent Office Group 162. From decision rejecting claims 24 to 27, applicant appeals. Affirmed.

DONALD R. DUNNER and LANE, AITKEN, DUNNER & ZIEMS (RONALD P. KANANEN of counsel) all of Washington, D. C., for appellant.

S. WM. COCHRAN (FRED E. McKELVEY of counsel) for Commisioner of Patents.

Before RICH, ALMOND, BALDWIN, and LANE. Associate Judges, and RE, Judge United States Customs Court, sitting by designation.

ALMOND, Judge.

This is an appeal from the decision of the Patent Office Board of Appeals affirming the rejection of claims 24-27 of appellant's application entitled "Dry Transfer Sheet and Method."[1] No claims have been allowed.

The invention relates to a dry transfer sheet having a plurality of discrete characters or indicia spaced apart from one another in order to enable independent and selective transfer of the individual characters. The elements of the dry transfer sheet are shown in Fig. 2:

Fig. 2.
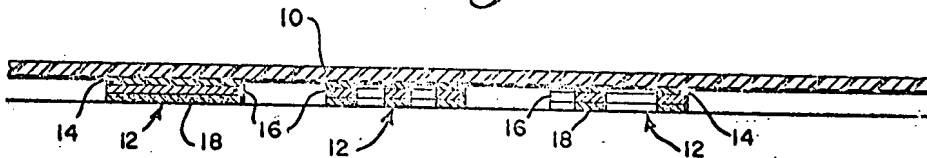
A light transmissive carrier sheet (10) supports a plurality of discrete reverse-printed characters (12) spaced apart from one another. In Fig. 2 the characters are composed of three layers. The first layer is a release layer (14); the second is an opaque film (16); and the third is a nonwaxy substantially nontacky pressure-sensitive adhesive (18). Alternatively, the opaque pigment may be incorporated into the release layer or into the adhesive, in which case a separate opaque film (16) is unnecessary. All layers of the characters may be silk screened onto the carrier, and they are to be in substantial registration while the remainder of the carrier sheet not covered by characters remains uncoated. During use, the individual characters are transferred by positioning the transfer sheet over the intended surface, with the adhesive layer adjacent said surface, and then rubbing against the front surface of the carrier sheet over the respective characters.

A single independent claim is on appeal:

24. A dry transfer sheet of the type which has a plurality of characters thereon and which is used by placing the sheet over

a layout, aligning a desired character with a specific location on the layout, then rubbing the sheet over the desired characters to transfer that character only to the layout without disturbing the remainder of the characters on the sheet, which comprises:

(a) a light transmissive carrier sheet;

(b) a plurality of discrete characters spaced apart from one another and adhered to the rear surface of said sheet, each character being capable of independent transfer to a layout from said sheet and each character comprising an opaque printed film member having

    i. a layer of a release agent immediately against the said rear surface, and

    ii. a layer of non-waxy substantially nontacky adhesive covering said layer of release agent and adapted to be engaged against the layout when the character is transferred;

(c) said printed film member having said layers in substantial registration and

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[1] Serial No. 300,743 filed August 8, 1963.

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Cl atoms having a marked anionic character.[30] This feature is also realized, but to a lesser extent, in the M–Cl bonds of complexes 2–6.

The present study illustrates for the first time the ability of an α-cyclodextrin cavity to recognize a transition metal M–Cl bond through weak Cl···H-5 interactions in the solid state as well as in solution. The fact that such subtle interactions could be observed in non-aqueous media is a consequence of the absence of stronger competing supramolecular forces, such as the hydrophobic effect, which usually plays a prevailing role in the formation of CD inclusion complexes. Overall these results illustrate the potential of modified cyclodextrins as second-sphere ligands.

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[25] Some insoluble material, presumably of oligomeric nature, was also formed during this synthesis. However, this is not the case for complexes 4–6 which were obtained from starting complexes containing very good leaving groups.

[26] Crystal structure analysis of 2·C₄H₈O: crystals suitable for X-ray diffraction were obtained by slow diffusion of pentane into a butanone solution of the complex. Crystal data: $M_r = 1782.98$, hexagonal, space group $P6_522$, $a = b = 14.8846(3)$, $c = 67.0615(15)$ Å, $V = 12867.0(5)$ Å³, $Z = 6$, $\rho_{calcd} = 1.381$ g cm⁻³, $Mo_{K\alpha}$ radiation $(\lambda = 0.71073$ Å), $\mu = 0.395$ mm⁻¹. Data were collected on a Bruker SMART 1000 CCD system at 133(2) K. The structure was solved by direct methods and refined on F_o^2 by full-matrix least squares (program SHELXL-97, G. M. Sheldrick, University of Göttingen). All non-hydrogen atoms were refined anisotropically; hydrogen atoms were included using a

riding model. The absolute configuration (and thus the enantiomeric space group assignment) was determined by a Flack x parameter of $-0.07(3)$. Refinement proceeded to $wR2 = 0.1025$ for all 5529 reflections and $R1 = 0.0413$ for data with $I > 2\sigma(I)$. The compound crystallizes with a butanone molecule positioned inside the cyclodextrin cavity. CCDC-181579 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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[28] Crystal structure analysis of 6·C₆H₆: crystals suitable for X-ray diffraction were obtained by slow diffusion of pentane into a benzene solution of the complex; $M_r = 1839.68$, orthorhombic, space group $P2_12_12_1$, $a = 15.353(2)$, $b = 24.313(2)$, $c = 26.952(3)$ Å, $V = 10060.9(19)$ Å³, $Z = 4$, $\rho_{calcd} = 1.211$ g cm⁻³, $Mo_{K\alpha}$ radiation $(\lambda = 0.71073$ Å), $\mu = 0.311$ mm⁻¹. Data were collected on a Kappa CCD Enraf Nonius system at 173(2) K. The structure was solved by direct methods and refined on F_o^2 by full-matrix least squares (program SHELXL-97, G. M. Sheldrick, University of Göttingen). All non-hydrogen atoms were refined anisotropically; hydrogens were included using a riding model. The absolute structure was determined by refining Flack's x parameter $(x = -0.01(4))$. $R1 = 0.0830$ and $wR2 = 0.2096$ for 12 112 data with $I > 2\sigma(I)$. The compound crystallizes with a benzene molecule included inside the cyclodextrin cavity. CCDC-181578 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

[29] The behavior of the CD cavity towards the "RuCl₂(CO)₂" unit strongly contrasts with that of a recently reported *p-tert*-butylcalix[4]-arene-derived diphosphane. Upon complexation, the latter favors inclusion of a "Ru–CO" rod over that of a "Ru–Cl" fragment; see reference [6].

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A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes**

Vsevolod V. Rostovtsev, Luke G. Green, Valery V. Fokin,* and K. Barry Sharpless*

Huisgen 1,3-dipolar cycloadditions[1] are exergonic fusion processes that unite two unsaturated reactants and provide fast access to an enormous variety of five-membered hetero-

[*] Prof. V. V. Fokin, Prof. K. B. Sharpless, Dr. V. V. Rostovtsev,
Dr. L. G. Green
Department of Chemistry and
the Skaggs Institute for Chemical Biology
The Scripps Research Institute, BCC-315
10550 N. Torrey Pines Rd., La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-7562
E-mail: fokin@scripps.edu, sharpless@scripps.edu

cycles.[2] The cycloaddition of azides and alkynes to give triazoles[3] is arguably the most useful member of this family. However, likely because of concerns about the safety of working with organic azides, synthetic chemists, in both pure and applied fields, have not given this transformation the special attention it deserves. Although the actual cycloaddition step may be faster and/or more regioselective for 1,3-dipoles other than azide, the latter is by far the most convenient to introduce and to carry hidden through many synthetic steps. Indeed, it appears to be the only three-atom dipole which is nearly devoid of side reactions.

Azides usually make fleeting appearances in organic synthesis: they serve as one of the most reliable means to introduce a nitrogen substituent through the reaction $-R-X \rightarrow [R-N_3] \rightarrow R-NH_2$. The azide intermediate is shown in brackets because it is generally reduced straightaway to the amine.[4] Despite this "azidophobia", we have learned to work safely with azides because they are the most crucial functional group for click chemistry endeavors.[5] Ironically, what makes azides unique for click chemistry purposes is their extraordinary stability toward H_2O, O_2, and the majority of organic synthesis conditions.[6] The spring-loaded nature of the azide group remains invisible unless a good dipolarophile is favorably presented.[7] However, even then the desired triazole-forming cycloaddition may require elevated temperatures and, usually results in a mixture of the 1,4 and 1,5 regioisomers [Eq. (1)].[1, 8]

ca. 1 : 1
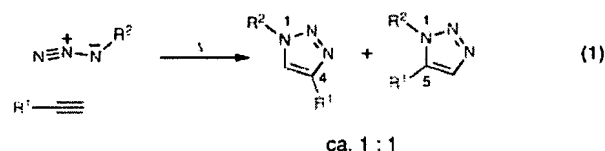
Since efforts to control this 1,4- versus 1,5-regioselectivity problem have so far met with varying success,[9] we were pleased to find this copper(I)-catalyzed reaction sequence which regiospecifically unites azides and terminal acetylenes to give only 1,4-disubstituted 1,2,3-triazoles.[10] The process is experimentally simple and appears to have enormous scope.

While a number of copper(I) sources can be used directly (see below), we found that the catalyst is better prepared in situ by reduction of Cu^{II} salts, which are less costly and often purer than Cu^{I} salts ($CuSO_4 \cdot 5H_2O$ serves well). As the reductant, ascorbic acid and/or sodium ascorbate proved to be excellent[11] for they allow preparation of a broad spectrum of 1,4-triazole products in high yields and purity at 0.25–2 mol% catalyst loading. The reaction appears to be very forgiving and does not require any special precautions. It proceeds to completion in 6 to 36 hours at ambient temperature in a variety of solvents, including aqueous tert-butyl alcohol or ethanol and, very importantly, water with no organic co-solvent.[12] Although most experiments were performed at near neutral pH values, the catalysis seems to proceed well at pH values ranging from approximately 4 to 12. In other words, this is a very robust catalytic process, which is so insensitive to the usual reaction parameters as to strain credulity.[13]

The reaction between phenyl propargyl ether and benzylazide in the presence of 5 mol% of sodium ascorbate and 1 mol% of copper(II) sulfate in a 2:1 mixture of water and tert-butyl alcohol furnished the 1,4-disubstituted triazole product in 91% yield after stirring for eight hours at room temperature in a capped scintillation vial, but otherwise with no effort to exclude oxygen [Eq. (2)]. The regiochemistry of the product was established by NOE experiments and confirmed by an X-ray crystallographic analysis.[14] For comparison, the thermal reaction (neat, 92°C, 18 h) between these substrates gives both regioisomers in a ratio of 1.6:1 in favor of the 1,4 isomer.
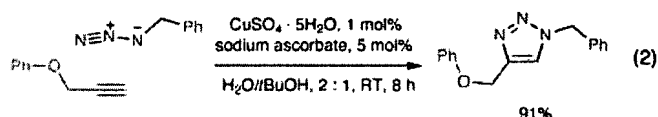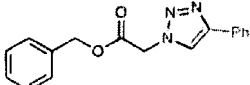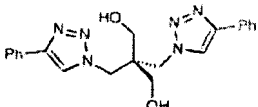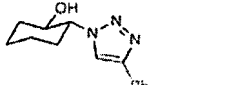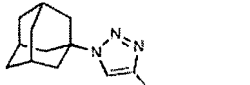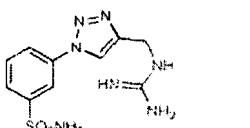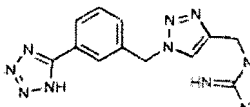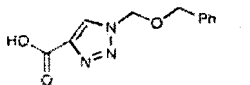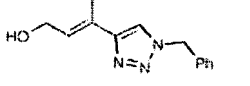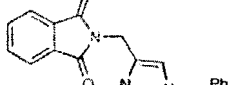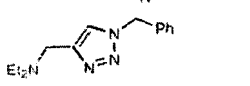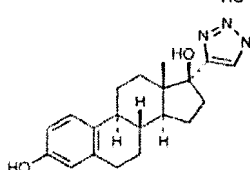
The scope of this copper-catalyzed triazole synthesis is partly revealed by the examples in Table 1; the lack of functional group interference is especially noteworthy. These triazoles are obtained by using a procedure which generally involves little more than stirring the reagents and filtering off pure products. Variously substituted primary, secondary, tertiary, and aromatic azides readily participate in this transformation. Tolerance for variations in the acetylene component is also excellent.

Copper(I) salts, for example, CuI, $CuOTf \cdot C_6H_6$, and $[Cu(NCCH_3)_4][PF_6]$, can also be used directly in the absence of a reducing agent. These reactions usually require acetonitrile as co-solvent and one equivalent of a nitrogen base (for example, 2,6-lutidine, triethylamine, diisopropylethylamine, or pyridine). However, formation of undesired by-products, primarily diacetylenes, bis-triazoles, and 5-hydroxytriazoles, was often observed.[10, 15] This complication with the direct use of Cu^{I} species was minimized when 2,6-lutidine was used, and exclusion of oxygen further improved product purity and yield.[16] Even though a broad range of both acetylene and azide components react readily in the acetonitrile system, we prefer the even more reliable and simple Cu^{II}/ascorbate aqueous system (with or without co-solvents and amine buffers/additives).

Our mechanistic proposal for the catalytic cycle is shown in Scheme 1. It begins unexceptionally with formation of the copper(I) acetylide I[17] (as expected, no reaction is observed with internal alkynes), but then gets interesting. Extensive density functional theory calculations[18] offer compelling evidence which strongly disfavors—by about 12–15 kcal—the concerted [2+3] cycloaddition (B-direct) and points to a stepwise, annealing sequence (B-1 →B-2 →B-3, hence the term "ligation"), which proceeds via the intriguing six-membered copper-containing intermediate III.[19]

In conclusion, the Cu^{I}-catalyzed transformation described here—a high-yielding and simple to perform "fusion" process leading to a thermally and hydrolytically stable triazole connection—is an ideal addition to the family of click reactions. The process exhibits broad scope and provides

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Entry	Product	Yield [%]
1		92
2		93
3		82
4		84
5		91
6		88
7		88
8		84
9		88
10		90
11		94

[a] All reactions were carried out in water with tert-butyl alcohol as co-solvent, 0.25–0.5 M in reactants, with 1 mol% of CuSO$_4$ and 10 mol% of sodium ascorbate, and were complete in 12–24 h.

1,4-disubstituted 1,2,3-triazole products in excellent yields and near perfect regioselectivity. The fact that this "unstoppable" reactivity[20] of copper(I) acetylides with organic azides remained unrevealed until now, despite the great body of research on copper-mediated organic synthesis over the last
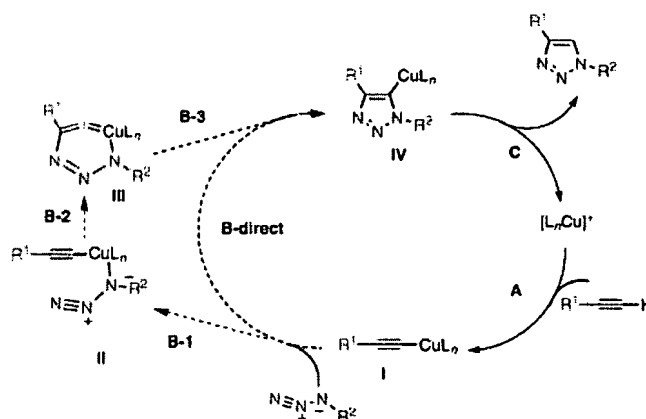
Scheme 1. Proposed catalytic cycle for the Cu^I-catalyzed ligation.

seventy years, is extraordinary. It can only be attributed to the often irrational fear of azides and is just another indication of the untapped potential of this underappreciated functional group. Above all, what this new catalytic process seems to offer is an unprecedented level of selectivity, reliability, and scope for those organic synthesis endeavors which depend on the creation of covalent links between diverse building blocks. Several studies which highlight the capabilities of the process, as well as studies hoping to achieve better mechanistic understanding of its unique reactivity features, are currently underway and will be reported in due course.

Experimental Section

General procedure (entry 11, Table 1): 17-ethynylestradiol (888 mg, 3 mmol) and (S)-3-azidopropane-1,2-diol (352 mg, 3 mmol) were suspended in a 1:1 mixture of water and tert-butyl alcohol (12 mL). Sodium ascorbate (0.3 mmol, 300 μL of freshly prepared 1M solution in water) was added, followed by copper(II) sulfate pentahydrate (7.5 mg, 0.03 mmol, in 100 μL of water). The heterogeneous mixture was stirred vigorously overnight, at which point it cleared and TLC analysis indicated complete consumption of the reactants. The reaction mixture was diluted with water (50 mL), cooled in ice, and the white precipitate was collected by filtration. After washing the precipitate with cold water (2 × 25 mL), it was dried under vacuum to afford 1.17 g (94%) of pure product as an off-white powder. M.p. 228–230°C. Elemental analysis calcd for C$_{23}$H$_{31}$N$_3$O$_4$: C 64.02, H 7.71, N 9.74%; found: C 64.06, H 7.36, N 9.64%. ^{1}H NMR ([D$_6$]DMSO) δ = 8.97 (s, 1H), 7.77 (s, 1H), 6.95 (d, J = 8.3 Hz, 1H), 6.45 (dd, J = 8.3, 2.3 Hz, 1H), 6.41 (d, J = 2.3 Hz, 1H), 5.13 (m, 1H), 5.09 (d, J = 2.9 Hz, 1H), 4.83 (m, 1H), 4.46 (m, 1H), 4.21 (m, 1H), 3.81 (m, 1H), 3.26 (m, 1H), 2.67 (m, 2H), 2.35 (m, 1H), 2.08 (m, 1H), 1.96 (m, 1H), 1.89–1.77 (m, 3H), 1.63 (m, 1H), 1.48–1.12 (m, 6H), 0.91 (s, 3H), 0.74 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO) δ = 154.8, 153.8, 137.2, 130.4, 126.0, 123.3, 114.9, 112.7, 81.1, 70.6, 70.4, 63.2, 52.6, 47.5, 46.7, 43.2, 37.2, 32.6, 29.3, 27.2, 26.1, 23.6, 14.4 ppm.

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[2] a) W.-Q. Fan, A. R. Katritzky in *Comprehensive Heterocyclic Chemistry II, Vol. 4* (Eds.: A. R. Katritzky, C. W. Rees, E. F. V. Scriven), Pergamon, Oxford, **1996**, pp. 101–126; b) R. N. Butler in *Comprehensive Heterocyclic Chemistry II, Vol. 4* (Eds.: A. R. Katritzky, C. W. Rees, E. F. V. Scriven), Pergamon, Oxford, **1996**, pp. 621–678; c) K. Banert, *Chem. Ber.* **1989**, *122*, 911–918.

[3] a) R. Huisgen, *Pure Appl. Chem.* **1989**, *61*, 613–628; b) R. Huisgen, G. Szeimies, L. Moebius, *Chem. Ber.* **1967**, *100*, 2494–2507; c) W. Lwowski in *1,3-Dipolar Cycloaddition Chemistry, Vol. 1* (Ed.: A. Padwa), Wiley, New York, **1984**, chap. 5; d) J. Bastide, J. Hamelin, F. Texier, V. Q. Ven, *Bull. Soc. Chim. Fr.* **1973**, 2555–2579; J. Bastide, J. Hamelin, F. Texier, V. Q. Ven, *Bull. Soc. Chim. Fr.* **1973**, 2871–2887.

[4] Although applications which utilize the unique reactivity offered by the azide group itself are rare, delightful exceptions can be found the following works: a) P. Desai, K. Schildknegt, K. A. Agrios, C. Mossman, G. Milligan, J. Aube, *J. Am. Chem. Soc.* **2000**, *122*, 7226–7232; b) K. Banert, *Targets Heterocycl. Syst.* **1999**, *3*, 1–32; K. Banert, *Liebigs Ann./Recl.* **1997**, 2005–2018; c) W. H. Pearson, W.-K. Fang, *Isr. J. Chem.* **1997**, *37*, 39–46; d) J. Cao, M. C. T. Fyfe, J. F. Stoddart, *J. Org. Chem.* **2000**, *65*, 1937–1946; e) B. Carboni, A. Benali, M. Vaultier, *J. Org. Chem.* **1993**, *58*, 3736–3741, and references therein.

[5] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 1198–1220; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.

[6] Organic azides, particularly in the aliphatic series, are exceptionally stable toward the common reactive chemicals on the Earth's surface, which range from dioxygen and water to the aqueous solutions of highly functionalized organic molecules which make up living cells: E. Saxon, C. R. Bertozzi, *Science* **2000**, *287*, 2007–2010; K. L. Kiick, E. Saxon, D. A. Tirrel, C. R. Bertozzi, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 19–24.

[7] In fact, it was the razor sharp reactivity window for this cycloaddition process which spawned our "in situ click chemistry" ideas—an approach which recently resulted in the discovery of the most potent noncovalent inhibitor of acetylcholinesterase known to date: W. G. Lewis, L. G. Green, F. Grynszpan, Z. Radic, P. R. Carlier, P. Taylor, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 1095–1099; *Angew. Chem. Int. Ed.* **2002**, *41*, 1053–1057.

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[12] The starting materials do not need to be dissolved in the reaction solvent. The reaction seems to proceed just as efficiently as long as adequate stirring is maintained.

[13] Amazingly, even Cu⁰ can be used as a source of the catalytic species. Although these reactions may take longer to proceed to completion, the experimental procedure is exceedingly simple. For example, bistriazole shown in entry 2 (Table 1) was obtained in quantitative yield after stirring the corresponding azide and acetylene components for 24 h with about 1 g of coiled copper metal turnings. The turnings were removed at the end of the reaction, and the pure white product was collected by simple filtration.

[14] CCDC 186236 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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[16] For example, ethyl propiolate and benzylazide furnished the corresponding 1,4-triazole in 55% yield when this procedure was used, but only a trace amount of the product was obtained with one equivalent of triethylamine and without exclusion of oxygen.

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Electronic and Steric Effects on Catalysts for CO₂/Epoxide Polymerization: Subtle Modifications Resulting in Superior Activities**

David R. Moore, Ming Cheng, Emil B. Lobkovsky, and Geoffrey W. Coates*

Carbon dioxide is an attractive chemical feedstock. In addition to the fact that CO_2 is an inexpensive substance of practically inexhaustible supply, it is also nonflammable and exhibits low toxicity.[1–3] Since CO_2 is believed to contribute significantly to global warming, its removal from the atmosphere has added environmental benefits. Consequently, the alternating copolymerization of carbon dioxide with epoxides to aliphatic polycarbonates has been a topic of increasing

[*] Prof. G. W. Coates, D. R. Moore, M. Cheng, E. B. Lobkovsky
Department of Chemistry and Chemical Biology
Baker Laboratory, Cornell University
Ithaca, NY 14853-1301 (USA)
Fax: (+1) 607-255-4137
E-mail: gc39@cornell.edu

Accelerated Bioorthogonal Conjugation: A Practical Method for the Ligation of Diverse Functional Molecules to a Polyvalent Virus Scaffold

Sayam Sen Gupta,[†] Jane Kuzelka,[†] Pratik Singh,[‡] Warren G. Lewis,[†] Marianne Manchester,[‡] and M. G. Finn*,[†]

Department of Chemistry and The Skaggs Institute for Chemical Biology, and Department of Cell Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037. Received May 23, 2005; Revised Manuscript Received August 12, 2005

Covalent bond formation to proteins is made difficult by their multiple unprotected functional groups and normally low concentrations. A water-soluble sulfonated bathophenanthroline ligand (2) was used to promote a highly efficient Cu[I]-mediated azide—alkyne cycloaddition (CuAAC) reaction for the chemoselective attachment of biologically relevant molecules to cowpea mosaic virus (CPMV). The ligated substrates included complex sugars, peptides, poly(ethylene oxide) polymers, and the iron carrier protein transferrin, with routine success even for cases that were previously resistant to azide—alkyne coupling using the conventional ligand tris(triazolyl)amine (1). The use of 4—6 equiv of substrate was sufficient to achieve loadings of 60—115 molecules/virion in yields of 60—85%. Although it is sensitive to oxygen, the reliably efficient performance of the Cu·2 system makes it a useful tool for demanding bioconjugation applications.

INTRODUCTION

Chemoselective ligation, a process whereby unprotected molecular fragments couple through uniquely complementary reactive functional groups, is a powerful technique for bioconjugation (1, 2). The azide group in particular has emerged as a very useful chemical handle for this purpose, participating in the Staudinger ligation with phosphines (1) as well as the [3 + 2] cycloaddition reaction with strained (3) and terminal (4) alkynes. Under the influence of copper(I) catalysis (4), the latter process has permitted the selective modification of virus particles (5, 6), enzymes (7), cells (8), and proteins from complex tissue lysates (9). The unreactive nature of both azides and alkynes with the functional groups of biomolecules, as well as their small size and therefore unobtrusive presence when appended to enzymatic substrates, makes improved methods for their ligation of interest for a wide range of biomolecular applications.

The successful execution of the Cu-catalyzed azide—alkyne cycloaddition (CuAAC) reaction at mild temperatures and low concentrations requires the presence of a copper-binding ligand. The ligand must accelerate the reaction, maintain the +1 oxidation state of the metal, and preserve the integrity of the biological scaffold by sequestering the copper ions away from proteins and polynucleotides. To this end, tris(triazolyl)amine ligand (1) (10) has been utilized for the conjugation of a variety of molecules to protein surfaces. Although 1 strongly accelerates the CuAAC process when the reaction is homogeneous, the ligand is not very soluble in water, and there have been some indications that proteins may be damaged if insufficient amounts of the ligand are avail-

able in solution (3). Most importantly, the reaction in water in the presence of 1 is too slow to eliminate the need for large excesses of substrate, on the same order of concentrations as are needed for the more conventional reactions of cysteine residues with maleimides. In other words, bioconjugation reactions catalyzed by Cu·1 are too demanding of precious or expensive coupling partners to be routinely useful for preparative applications.

1   **2**
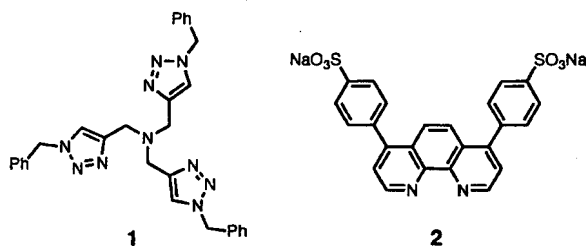
For several years, we have developed the use of icosahedral virus particles as chemical building blocks for studying the role of polyvalency in diagnostic and therapeutic applications (11, 12). As a prototypical scaffold, we have employed cowpea mosaic virus (CPMV), which is a stable and crystallographically characterized particle available in large quantities. The range of target functions is broad, requiring the attachment of such structures as carbohydrates, peptides, organic polymers, and proteins. A single common attachment methodology would greatly assist synthetic access to diverse particles. However, the aforementioned deficiencies of the Cu·1 system constrained our initial efforts to obtain such materials in relatively large amounts. We describe here an improved procedure for the CuAAC process that makes it a truly general method for polyvalent scaffold decoration and, by extension, for the creation of functionalized surfaces of almost any kind. Importantly, this

* To whom correspondence should be addressed. E-mail: mgfinn@scripps.edu.
† Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute.
‡ Department of Cell Biology, The Scripps Research Institute.

system permits the chemoselective bioconjugation of molecules that were hitherto unreactive to azide—alkyne coupling.

EXPERIMENTAL PROCEDURES

Materials. Fluorescein-PEG-NHS-3400 (NHS = N-hydroxysuccinimide) was obtained from Nektar (Huntsville, AL). The water-soluble sulfonated bathophenanthroline ligand **2** was purchased from GFS. Human holotransferrinn (98%) was supplied by Sigma. The resins Fmoc-Phe-Wang (0.77 mmol/g, 100—200 mesh) and Fmoc-Lys(Boc)-Wang (0.12 mmol/g, 100—200 mesh), as well as other Fmoc-protected amino acids were purchased from Chem-Impex International. Compounds **5** (*13*), **6** (*13*), and [Cu(MeCN)$_4$](OTf) (*14*) were prepared as previously described; **7a** and **8a** were provided by the Consortium for Functional Glycomics at The Scripps Research Institute. CPMV—alkyne and —azide conjugates **3** and **4** were prepared as previously described (*13*) using purified NHS esters of the acid-bearing linkers. Fmoc-L-propargylglycine was purchased from CSPS (San Diego, CA). All other chemical reagents were obtained from commercial suppliers and used as received, unless indicated otherwise.

Instrumentation. Air-sensitive manipulations were performed under nitrogen in a Vacuum Atmospheres glovebox. Preparative HPLC was performed with a Dynamax/Rainin Preppy SD-1 instrument and a Vydac protein and peptide reverse phase column, eluting with a gradient solvent mixture (solvent A = H$_2$O/0.1% TFA; solvent B = CH$_3$CN/0.1% TFA). MALDI-TOF analyses were performed by the Mass Spectrometry Facility at The Scripps Research Institute. FPLC analyses were performed on an AKTA Explorer (Amersham Pharmacia Biotech) equipped with a Superose-6 size exclusion column. Samples for transmission electron microscopy (TEM) were obtained by deposition of 20 μL sample aliquots onto 100-mesh carbon-coated copper grids, followed by staining with 20 μL of 2% uranyl acetate. Images were obtained using a Philips CM100 electron microscope.

Modification of CPMV with NHS Esters. Reagents were introduced into a solution of CPMV, such that the final mixture contained ≤20% DMSO. Unless otherwise specified, the buffer used was 0.1 M phosphate, pH 7.0. Purification of derivatized virus (>1 mg) was performed by ultracentrifugation over a 10—40% sucrose gradient, pelleting of the recovered virus, and dissolution of the resulting material in Tris buffer (0.1 M, pH 8). Mass recoveries of derivatized viruses were typically 60—80%; all such samples were composed of >95% intact particles as determined by analytical size-exclusion FPLC. Virus concentrations were measured by absorbance at 260 nm; virus at 0.10 mg/mL gives a standard absorbance of 0.80. Fluorescein concentrations were obtained by measurement of absorbance at 495 nm, applying an extinction coefficient of 70 000 M^{-1} cm^{-1}. Each data point is the average of values obtained from three independent parallel reactions; loading values (the number of substrate molecules attached to the virus) are subject to an experimental error of ±10%. The average molecular weight of the CPMV virion is 5.6 × 10^6 g/mol.

Compounds 7b and 8b. To a solution of **7a** (10 mg, 12.4 μmol) in H$_2$O (1 mL) was added **9** (70 mg, 0.125 mmol) in THF (1 mL). *t*-BuOH (1 mL) was added, followed by sodium ascorbate (0.5 M in H$_2$O, 72 μL, 36 μmol) and CuSO$_4$ (0.5 M in H$_2$O, 24 μL, 12 μmol). The reaction mixture was stirred in a closed vial for 48 h at room temperature, followed by removal of the volatile

solvents by rotary evaporation and addition of 5 mL of H$_2$O. Excess **9** was largely removed by extraction with EtOAc. The reaction was monitored by TLC (R_f = 0.6 in 8:3:3:2 EtOAc/MeOH/AcOH/H$_2$O,) as well as by disappearance of the azide peak (2100 cm^{-1}) using FT-IR spectroscopy. The aqueous phase was concentrated by evaporation and residual **9** was removed by column chromatography (Sephadex G-15, 95:5 H$_2$O/BuOH), giving a yellow solid (11 mg, 65% yield) upon lyophilization of the collected fraction. MALDI-TOF: [M + H]$^+$ = 1361, [M + Na]$^+$ = 1383, [M + K]$^+$ =1399. Compound **8b** was synthesized in 55% yield from **8a** using the same procedure. MALDI-TOF: [M + Na]$^+$ = 1472, [M + K]$^+$ = 1488.

Compound 9. A mixture of fluorescein amine (1.57 g, 4.54 mmol) and sodium bicarbonate (1.57 g, 1.87 mmol) in dry THF (30 Ml) was cooled in an ice bath and stirred under N$_2$ for 15 min. 3,5-Bis-prop-2-ynyloxy-benzoyl chloride (1.15 g, 4.99 mmol) in dry THF (30 mL) was added dropwise and the mixture was stirred overnight at room temperature. The solid bicarbonate was removed by filtration, and the solvent was evaporated to give **4** as an orange solid, which was purified by column chromatography (silica gel, 95:5 EtOAc/MeOH). ^{1}H NMR (CD$_3$OD, δ) 8.4 (s, 1H), 8.2 (d, 2H), 7.3 (m, 3H), 6.8—7 (m, 3H), 6.6—6.8 (m, 4H), 4.8 (d, 4H) (s, 6 H), 3.1 (t, 2H). ESI-MS m/z = 560.1 (MH$^+$); UV—vis (0.1 M phosphate, pH 7) λ_{max} 494 nm, ϵ = 64 000.

Peptides 10 and 11. Compound **10** was prepared by standard techniques of solid-phase Fmoc peptide synthesis using 0.2 mmol of Fmoc-Phe-Wang resin. Coupling of Fmoc-L-propargylglycine was performed as reported elsewhere (*15*). Conjugation of fluorescein to the N-terminus of the peptide chain was accomplished by addition of a DMF/*i*Pr$_2$Net (2:1 v/v) solution containing 5(6)-carboxyfluorescein (414 mg, 1.1 mmol) and HBTU (417 mg, 1.1 mmol) to the drained resin. The mixture was agitated overnight and purified by reverse phase HPLC after cleavage from the resin. MALDI-TOF: [M + H]$^+$ = 1579. Peptide **11** was obtained from the analogous procedure using 0.1 mmol of Fmoc-Lys(Boc)-Wang resin. MALDI-TOF: [M + H]$^+$= 1571, [M + Na]$^+$ = 1593.

Polymer 12. A toluene solution of 3-azido-1-propylamine (0.66 M, 334 μL, 0.22 mmol) was added to a solution of fluorescein-PEG-NHS-3400 (150 mg, 0.044 mmol) in dry CH$_2$Cl$_2$ (5 mL). The mixture was stirred overnight, followed by removal of the solvents under reduced pressure. H$_2$O (10 mL) was added and the solution was extracted with EtOAc to remove the excess azide compound. The aqueous solution was lyophilized to afford **12** as a yellow powder (135 mg, 90% yield).

Polymer 13. To a solution of fluorescein-PEG-NHS-3400 (150 mg, 0.044 mmol) in dry CH$_2$Cl$_2$ (5 mL) was added propargylamine (12.1 mg, 0.22 mmol). The mixture was stirred overnight and worked up as described for **12**. Compound **13** was isolated as a yellow powder (135 mg, 90% yield).

Transferrin—Alkyne Conjugate 14. To human holo-transferrin (50 mg, 0.625 μmol) in phosphate buffer (0.1 M, pH 7, 2 mL) was added N-(N-(prop-2-ynyl)hexanamidyl)maleimide (3.9 mg, 9.1 μmol) in DMSO (500 μL), and the mixture was incubated overnight at room temperature. Purification through a G-15 Sephadex coloumn followed by dialysis and lyophilization afforded **14** as a pink powder (30 mg).

Modification of CPMV by CuAAC Reaction. CPMV conjugate **3** or **4** (1 mg as a 2 mg/mL solution) was incubated with complementary azide or alkyne compound (concentrations given in Table 1) in Tris buffer (0.1 M, pH 8, 0.5 mL) containing **2** (3 mM) and [Cu(MeCN)$_4$](OTf)

Scheme 1
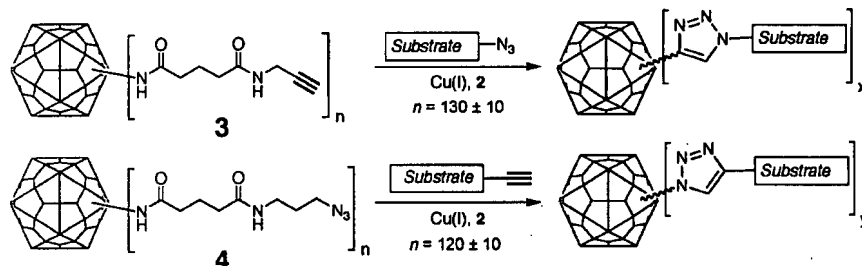
Table 1. Azide−Alkyne Cycloaddition on CPMV[a] with Various Substrates

entry	substrate	CPMV derivative	[substrate] (μM)	loading	yield (%)
1	7b	4	200	115	85
2	8b	4	200	105	85
3	10	4	120	60	85
4	11	4	250	115	80
5	12	3	500	60	60
6	13	4	250	90	75
7	14	4	260	b	b

[a] 2 mg/mL; 47 μM in alkyne for 3, 43 μM in azide for 4. [b] not determined.

(1 mM) for 12 h at room temperature with rigorous exclusion of dioxygen. CPMV−12, CPMV−13, and CP-MV−14 conjugates were purified by sucrose gradients and pelleting as described above. All other CPMV conjugates were purified by size exclusion chromatography using Bio-Spin disposable chromatography columns filled with Bio-Gel P-100 as described elsewhere (*16*).

RESULTS AND DISCUSSION

Optimization of Reaction Conditions. Our recent discovery of sulfonated bathophenanthroline 2 as a highly efficient ligand in a fluorescence quenching catalysis assay (*17*) prompted us to further investigate 2 for the coupling of compounds to suitably derivatized CPMV particles. The viral capsids were labeled with alkynes (3) or azides (4) at surface-exposed lysine residues using standard NHS ester chemistry (Scheme 1) (*5*). Initial experiments were performed using functionalized fluorescein dyes as substrates because the success of the bioconjugation could be readily monitored using UV−vis spectroscopy. Thus, fluorescein derivatives 5 and 6 (Figure 1) (*5*) were condensed with 3 and 4, respectively, in the presence of Cu·2 in Tris buffer (pH 8) under inert atmosphere, to give CPMV−dye conjugates with good loading in a concentration-dependent fashion. In all cases, the reaction yield (the percent of virus recovered after purification of protein away from small molecules) and purity (intact virus particles vs disassembled viral protein) was high. Thus, >85% of the protein was recovered in each case, and size-exclusion FPLC indicated that >95% of the virons were intact particles (*18*). SDS−PAGE analysis visualized under ultraviolet light revealed two dye-labeled bands corresponding to the small and large subunits of CPMV, indicating that both subunits of the virus were chemically modified (data not shown). No attachment was found to occur in the absence of Cu[I], ruling out nonspecific adsorption of dye to virus. It should also be noted that the use of phosphate buffer diminishes the effectiveness of the reaction, while HEPES buffer is at least as good or better than Tris.

The dependence of the observed loading (dye attachments per virion) on substrate concentration is shown in Figure 2. Upon treatment of 2 mg/mL 4 (0.36 μM in virus particles) with 200 μM 6, corresponding to a 5-fold molar excess with respect to azide groups on 4, the CPMV particles were found to be fully labeled (in other words, all of the attached azide groups converted to triazoles, approximately 110 per particle). Similar results were obtained with 3 + 5. In contrast, the use of ligand 1 under otherwise identical conditions required a 5 mM concentration of 6 (250 equiv) to achieve such dye loadings. Furthermore, the reaction of opposite "polarity" (3 + 5) mediated by Cu·1 was significantly worse than 4 + 6.

The same results with each ligand were obtained using [Cu(MeCN)₄](OTf), [Cu(MeCN)₄](PF₆), or CuBr as the source of Cu[I]. The optimal copper concentration was found to be 1 mM; lower concentrations significantly decreased the coupling efficiency. The ligand-to-metal ratio is also important. A 3:1 ratio of 2 to Cu[I] afforded the best results; a lower ratio resulted in significant degradation of the viral capsid, whereas a larger excess of ligand slowed the reaction to provide incomplete labeling. The efficiency of the Cu·2 mediated AAC process thereby far exceeds that of standard NHS and maleimide coupling reactions with lysine and cysteine side chains, respectively. For example, the addition of a 10-fold excess of fluorescein NHS ester to CPMV under similar conditions results in the attachment of approximately 20 dyes to each capsid (*19*), and fluorescein maleimide deposits between 10 and 25 dye molecules on CPMV mutants bearing surface cysteine residues, depending on the local environment of the sulfhydryl groups (*20*). While the linker used to attach azides and alkynes to CPMV may make these groups more accessible than the lysine or cysteine side chains of native and mutant forms of the particle, we believe that the differences will be small given the highly solvent-exposed nature of many of the surface peptide residues.

Preparation of CPMV−Carbohydrate Conjugates. With the optimal reaction conditions thus established, biologically relevant substrates were attached to the CPMV capsid as shown in Figure 1 and Table 1. Carbohydrate 7a binds the protein galectin-4, an early marker of breast cancer cells (*21*). Sialyl Lewis X, an azide derivative of which is 8a, is overexpressed on cancer cells and also plays a role in inflammation (*22*). We envisioned that the attachment of these two compounds to the surface of a virus particle could be useful for drug targeting, as well as for the elusive goal of antibody production against carbohydrate epitopes (*23*). To allow for ready quantitation of the attachment of these nonfluorescent compounds, the azides were subjected to a CuAAC reaction with fluorescein dialkyne reagent 9 to provide dye−alkyne derivatives 7b and 8b. Using the Cu·2 system, 7b and 8b were then successfully grafted to virus−azide 4 with loadings of 115 and 105 per virion, respectively. Only 4 equiv of 7b or 8b per azide group on 2 was necessary to reach this level of loading at a virus
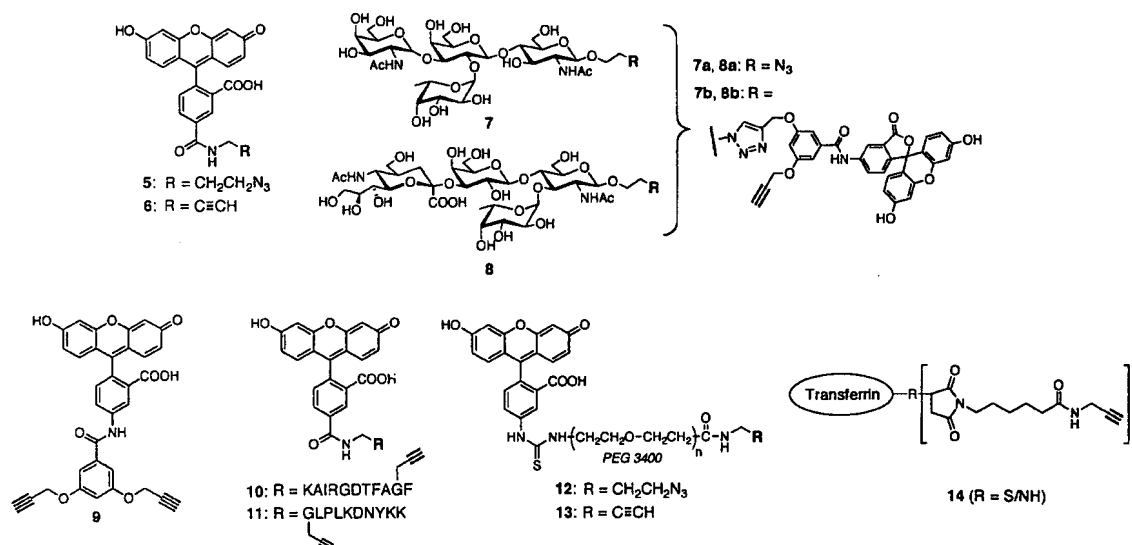
Figure 1. Substrates used in CuAAC attachment to CPMV.
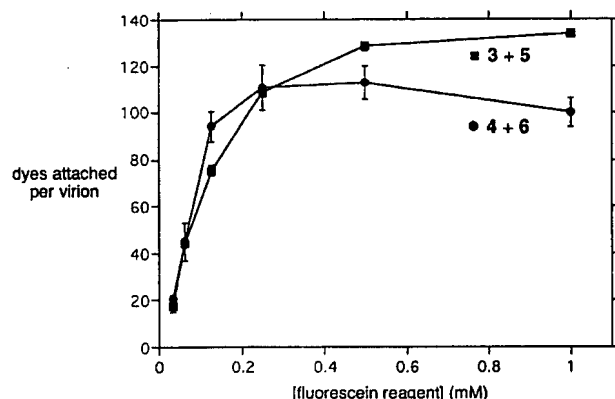
Figure 2. Dependence of dye loading on reagent concentration. Conditions used: 2 mg/mL **3** or **4**, complementary fluorescein derivatives **5** or **6**, 1 mM [Cu(MeCN)$_4$](Otf), 3 mM **2**, Tris-HCl buffer (pH 8), r.t., 14 h.

concentration of 1–2 mg/mL. The integrity of polyvalently displayed **7** and the retention of the activity of the carbohydrate was verified by the formation of a gel upon the addition of CPMV–(**7b**)$_{115}$ to dimeric galectin-4 (*18*). The use of **7a** and **8a** with particle **3** under similar conditions likewise gave intact derivatized virions in high yield with the ability to efficiently crosslink a solution of galectin-4. We assume in these cases that the loading of small molecules lacking the fluorescein tag is approximately the same as for their fluorescent counterparts since we have established with extensive studies that the nature of the substrate has little effect on the efficiency of the CuAAC reaction.

This facile attachment of complex, unprotected sugars to proteins by CuAAC ligation represents a significant advancement over existing methodologies employing a bifunctional linker on the carbohydrate for standard bioconjugation reactions. Typically, squarates (*24*) and maleiimide-hydrazide or maleiimide-NHS ester linkers have been employed for this purpose (*23*, *25*), and the additional synthetic steps required to functionalize the sugars in the appropriate fashion result in poor overall coupling yields. In contrast, azides can be readily incorporated into the carbohydrate scaffold early in the synthesis and rarely interfere in subsequent synthetic steps.

Attachment of Peptides to CPMV. Although the genetic incorporation of peptide loops into selected regions of the CPMV capsid structure is well established (*26*), the production of such chimeras suffers from restrictions in terms of size, position, and sequence. Given the great importance of cyclic and linear peptides to a wide variety of targets in biochemistry, molecular recognition, and drug development, robust methods for the attachment of natural and nonnatural oligopeptides to polyvalent scaffolds are of interest. To demonstrate the virtues of the CuAAC reaction in this regard, we chose peptides containing carboxylic acid or amine side chain functional groups and which would therefore require protection/deprotection strategies to be incorporated in standard peptide coupling procedures. To date, the decoration of full proteins with functional peptides has been accomplished predominantly with native chemical ligation (*27*) or maleimide-cysteine reactions. Both of these strategies require the presence of accessible cysteine residues in the protein, the former at the N-terminus (*28*).

We tested the Cu·**2** system with two functional peptides. The arginine-glycine-aspartate (RGD) sequence of **10** is derived from an adenovirus serotype that binds α$_v$ integrins, extracellular matrix receptors that are overexpressed on many cancer cells (*29*). The amino acid sequence of **11** comes from a portion of the protective antigen (PA) of anthrax toxin, a moiety that binds edema factor (EF) and lethal factor (LF) and permits cell entry of the toxin (*30*). Peptide **10** was successfully attached to **4** with a loading of 60 peptides per viral particle using only a 6-fold excess of substrate and standard Cu·**2** conditions. Significantly, no peptide attachment was obtained when ligand **1** was employed with up to 5 mM substrate present. The attachment of **11** afforded a loading of 115 peptides/virion, and SDS–PAGE analysis by UV irradiation indicated that both small and large subunits of CPMV were modified with the PA peptide (data not shown). The ready incorporation of alkyne groups into synthetic peptides (*15*) permits the Cu·**2**-mediated AAC reaction to serve as a general strategy for the attachment of peptides to biomolecular scaffolds.

Preparation of Virus–Polymer Constructs. CPMV was previously derivatized with poly(ethylene oxide) (PEG) using an NHS ester derivative to give well-controlled loadings of the polymer on the outer coat-
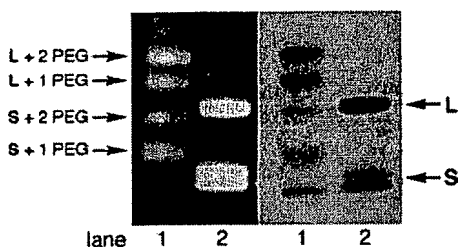
Figure 3. SDS−PAGE of CPMV−$(13)_{90}$ (lane 1) and CPMV−$(5)_{110}$ (lane 2). On the right (light background) is shown the gel visualized after SimplyBlue staining; the two extra bands corresponding to each subunit arise from modification by 1 or 2 PEG-3400 moieties. On the left (dark background) is the gel illuminated by UV light prior to protein staining. Because PEG-3400 is labeled with fluorescein, only the modified subunits are visible in lane 1. The two small-subunit bands appearing in lane 2 arise from incomplete C-terminal peptide cleavage in vivo and are unrelated to the present experiments.

protein assembly (*12*). Compared with wild-type CPMV, the PEGylated particle showed altered physical properties and a reduced immunogenic response in mice. Lysine reactivity with activated ester derivatives of PEG allowed us to reach a maximum of only 30 attached PEG molecules per virion. Attempts to boost the loading past this value required such high concentrations of PEG reagents that the virus particle precipitated before the reaction could occur. The enhanced activity of the Cu·2 catalyst allowed us to improve on this prior result. Thus, fluorescein end-functionalized PEG reagents **12** and **13** were coupled to their complementary CPMV alkyne and azide scaffolds to give loadings of 60 and 90 PEG chains

per virion, respectively, using easily accessible concentrations in which the virus particles were stable (Table 1). The resulting particles were again less dense on sucrose gradient sedimentation and larger as indicated by size-exclusion FPLC (*18*). Figure 3 shows the denaturing gel of CPMV−$(13)_{90}$ and CPMV−$(5)_{110}$ visualized by UV irradiation and protein staining. In both cases, both large (L) and small (S) subunits of the CPMV coat protein were labeled, as expected. The PEG conjugate CPMV−**13** gave rise to two higher molecular weight bands for each subunit, corresponding to single and double labeling of the subunits by the polymer. Protein staining of this conjugate also revealed the presence of a small proportion of unmodified subunits.

Attachment of the Transferrin Protein. As a final example of the ability of Cu·2 to efficiently promote the AAC reaction, the coupling of a large protein to the outer surface of CPMV was performed. Receptors for transferrin (Tfn), an iron carrier protein in vertebrates, are overexpressed on a variety of cancer cells. Polyvalent assemblies of Tfn on such scaffolds as liposomes and iron oxide nanoparticles have therefore been prepared for cancer cell targeting (*31*). The display of multiple copies of Tfn on CPMV could similarly afford a particle that binds tightly and selectively to receptor-bearing cells.

Human holo-transferrin, an 80 kDa bilobed glycoprotein, was incubated at high concentration (20 mg/mL) with 15 equiv of a maleimide-alkyne linker at pH 7 to afford the alkyne-derivatized protein **14**, with attachments made at one or more accessible cysteines (and perhaps, to a lesser extent, lysine) residues. The successful conjugation of alkyne to Tfn was verified by the
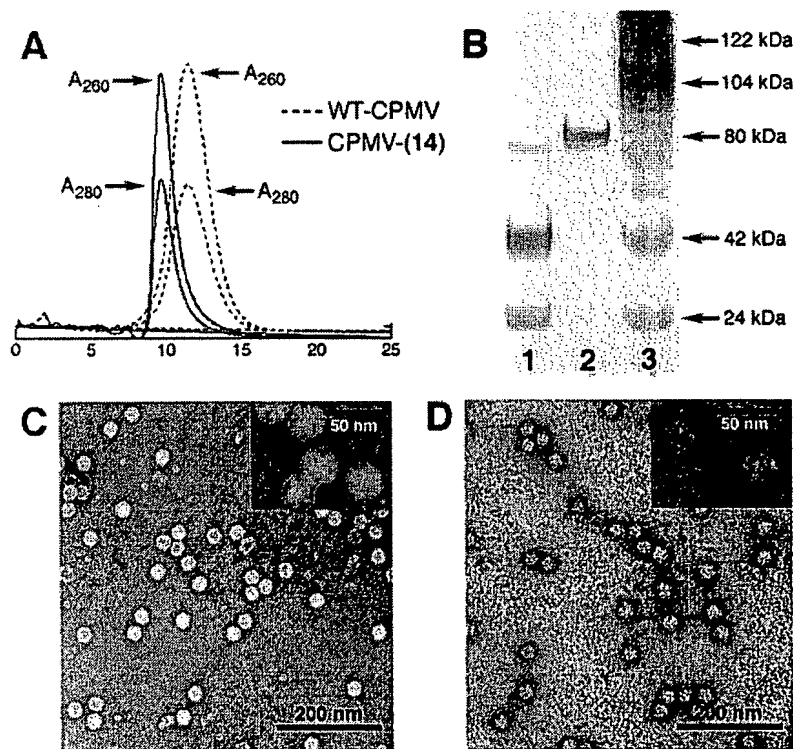
Figure 4. (A) Size-exclusion FPLC of wild-type CPMV and CPMV−$(14)_n$. Protein from disassembled particles would appear at longer retention times than the peaks observed here, and the A_{260}/A_{280} ratios are characteristic of intact, RNA-containing capsids for both samples. The more rapid elution of CPMV−$(14)_n$ indicates a substantial size increase in the particle, as 10 mL is approximately the void volume of the column. (B) SimplyBlue-stained gel (4−12% bis-tris) of wild-type CPMV (subunits at 42 and 24 kDa) (lane 1), Tfn (80 kDa) (lane 2), and CPMV−$(14)_n$ (lane 3). Note the appearance of two strong bands of approximately 102 and 122 kDa in the lane 3, corresponding to the CPMV subunits conjugated with Tfn. (C) Negative-stained TEM of wild-type CPMV. (D) Negative-stained TEM of CPMV−$(14)_n$. Automated measurement of the particles showed the average diameters to be 30 ± 1 nm for wild-type and 46 ± 5 nm for CPMV−$(14)_n$.

reaction of **14** with the fluorescein derivative **5** under CuAAC conditions. Analysis by SDS–PAGE confirmed that all of the Tfn was covalently labeled with at least one fluorescein molecule (data not shown).

The CPMV–Tfn conjugate CPMV–$(14)_n$ was then prepared by reaction of **4** with **14** using Cu·**2**. Examination of the product by FPLC, SDS–PAGE, TEM (Figure 4), and Western immunoblotting (*18*) indicated that a significant number of Tfn molecules were arrayed on the particle. Importantly, the virus–protein conjugates were isolated as individual particles, with no evidence of aggregation that might be expected if Tfn species bearing more than one alkyne were to couple to polyvalent CPMV azides. In negative-stained electron microscopy, individual CPMV–$(14)_n$ particles were larger than wild-type CPMV by approximately 16 nm in diameter, and displayed a clear knobby appearance contrasting with the smooth hexagonal shape of the wild-type virion. These observations confirm that Tfn molecules were covalently attached evenly over the CPMV surface. Preliminary measurements show the attached Tfn molecules to be active in binding the target receptor.

A recent report employing thiol-maleimide chemistry for the attachment of proteins (up to 22 kDa) to CPMV required the use of a 50-fold excess of protein with respect to the viral asymmetric unit, as well as subsequent nonchromatographic purification of the desired conjugate (*32*). We have obtained many similar results for NHS ester-lysine as well as thiol-electrophile reactions. In contrast, protein conjugation is achieved by the CuAAC reaction with efficiencies comparable to those of native chemical ligation (NCL) (*27*). Here, only a 6-fold excess of Tfn was required, and the relatively small amount of Tfn employed allowed for simple purification by sucrose gradients and pelleting. While NCL reactions are typically conducted with nearly equimolar ratios of coupling reagents, the concentrations of thioester and *N*-terminal cysteine reaction partners are typically much higher (0.1–1 mM) than the CPMV azides and alkynes used here (*33*). The Cu·**2**-mediated AAC protocol is therefore an excellent alternative for the coupling of suitably functionalized proteins (*34*).

In summary, we have developed a highly efficient azide–alkyne cycloaddition protocol using a simple copper(I) salt and sulfonated bathophenanthroline (**2**) for chemoselective ligation. This catalytic system permits the attachment of complex carbohydrates, peptides, polymers, and proteins to biomacromolecules in yields and substrate loadings far superior to those possible with previously established procedures. Advantages to the Cu·**2**-mediated AAC method include the use of modest excesses of the desired coupling partners and simple purification. The unfortunate tendency of copper ions to speed the hydrolytic cleavage of peptides and polynucleotides is largely controlled by the use of enough ligand to restrict access to the metal center. We anticipate that the improved CuAAC reaction will be particularly beneficial to those wishing to join substrates that are expensive or available in only small quantities and for biological molecules in which azides or alkynes are incorporated by biosynthetic procedures (*6, 35*). The single drawback to this system is the requirement that the reaction be performed under inert atmosphere; ligands designed to solve this problem are currently being developed.

ACKNOWLEDGMENT

Supporting Information Available: Size-exclusion FPLC of CPMV conjugates, reaction of CPMV-**8b** with dimeric galectin-4, Western blots of CPMV–$(14)_n$. This material is available free of charge via the Internet at http://pubs.acs.org.

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Synthesis of Symmetric and Unsymmetric Triazole Dendrimers via Dipolar Cycloaddition Reaction

Jae Wook Lee,* Byung-Ku Kim

Department of Chemistry, Dong-A University, Hadan-2-dong, Busan 604-714, Korea
Fax +82(51)2007259; E-mail: jlee@donga.ac.kr

Abstract: Symmetric and unsymmetric dendrimers containing 1,2,3-triazole rings at the core were synthesized via the 1,3-dipolar cycloaddition of Fréchet-type dendron-azides with dendron-acetylenic esters.

Key Words: 1,3-dipolar cycloaddition, dendrimer, 1,2,3-triazoles

Since the 1,3-dipolar cycloaddition of azides with alkynes was investigated by Huisgen et al.[1] it has attracted much attention because of the synthetic importance of the aromatic and nonaromatic five-membered 1,2,3-triazole heterocycles.[2] The traditional method for producing the triazole by cycloaddition requires elevated temperature, typically in refluxing conditions, and also provides a mixture of 1,4-disubstituted and 1,5-disubstituted triazoles. Over the years, several efforts to control the 1,4- versus 1,5-regioselectivity have been reported.[3] Recently, the Cu(I)-catalyzed Huisgen [2+3] dipolar cycloaddition reaction between an organic azide and a terminal alkyne, has attracted attention.[4] The reaction, characterized by very high yields, mild and simple reaction conditions, oxygen and water tolerance, and simple product isolations, is highly chemoselective affording only the desired 1,2,3-triazole, even in the presence of a large variety of other functional groups. The methodology has found many applications in organic chemistry,[5] supramolecular chemistry,[6] drug discovery,[7] bioconjugations,[8] and dendritic materials science.[9] Due to our interest in developing new dendrimers, we became involved in exploring an efficient cycloaddition reaction that provides an easy access to dendrimers.[10] Here we present the convergent synthesis of symmetric and unsymmetric triazole dendrimers by dimerization of dendrons via a [2+3] dipolar cycloaddition reaction between azides and acetylenes.

The synthetic strategy for triazole dendrimers **3-Gmn** utilized a convergent method using Fréchet-type azido dendrons **1-Dm** and acetylenic dendrons **2-Dn** (Figure 1). We selected the electron-deficient acetylenic dendron **2-Dn** because it is known that alkynes with an electron-withdrawing functional group favor the Huisgen cycloaddition of azides and alkynes. The Fréchet-type azido dendrons **1-Dm** (m = 1, 2, 3: generation of dendron)[11] and the acetylenic dendrons **2-Dn** (n = 1, 2, 3: generation of dendron)[10b]
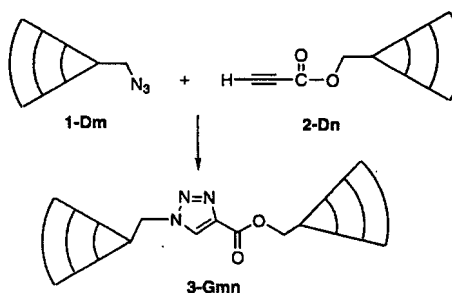
Figure 1 Synthetic strategies of triazole dendrimers **3-Gmn** by dimerization of dendrons

shown in Figure 2 were prepared according to the reported procedure.

To probe the viability of our approach, the coupling was initially studied using azido dendron **1-D1** and acetylenic dendron **2-D1** in toluene at different temperatures (Table 1, entries 1–3). The reaction conducted at higher temperatures (120 °C or 80 °C) provided the 1,2,3-triazole product in excellent yields in 2:1 or 2.7:1 ratio of 1,4- to 1,5-isomers, respectively. The reaction carried out at low temperature (50 °C) reduced the generation of the 1,5-isomer and provided the 1,2,3-triazole product in excellent yields in 9:1 ratio of 1,4- to 1,5-isomers. The results indicate that higher reaction temperatures not only increase product yields but also change the ratio of regioisomers in favor of the 1,5-isomer.

The amounts of 1,4- and 1,5-isomers in the reaction mixture were established after separation of the regioisomers by column chromatography and characterization by ^{1}H NMR spectra. The triazole proton of the 1,4-regioisomer **3-G11** was found at 7.98 ppm, whereas in the corresponding 1,5-isomer it was shifted downfield to 8.17 ppm. The isomer was confirmed by ROESY. The NOE effect was observed between the triazole proton and the N-substituted methylene proton in 1,4-regioisomer **3-G11**, suggesting that the triazole proton and the N-substituent are in close proximity as in the 1,4-substitued triazole.

Recently it was reported that Cu(I) species in Huisgen [2+3] dipolar cycloaddition reactions can be manipulated to provide the product regioselectively.[4] To better the regioselectivity and yield, we carried out the reactions in the presence of CuI as an additive (Table 1, entries 4–5). The reaction of **1-D1** with **2-D1** at 50 °C and at room temperature in toluene provided the 1,2,3-triazole product **3-G11**
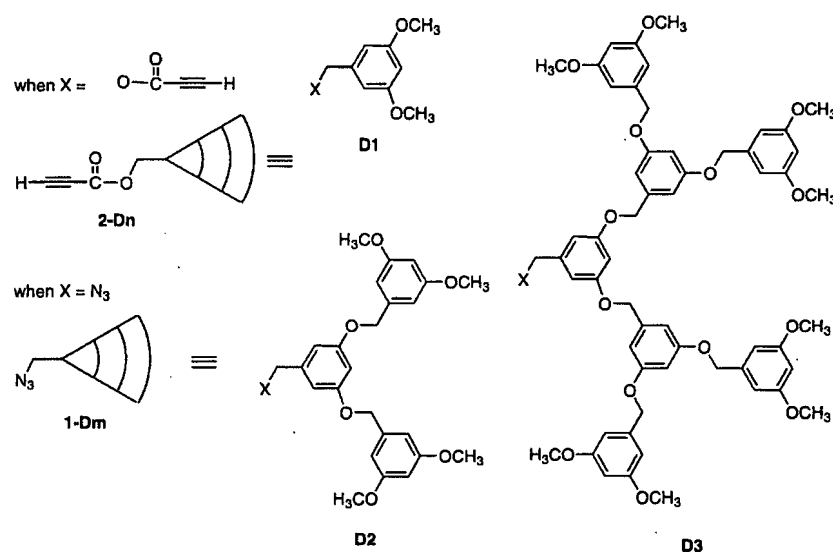
Figure 2 Structures of azido dendrons **1-Dm** and acetylenic dendrons **2-Dn**

in excellent yields with 20:1 and 22:1 ratios of 1,4- to 1,5-isomers, respectively.

Next, we investigated the triazole dendrimer synthesis from the reactions of **1-D2** with **2-D2** and of **1-D3** with **2-D3** in the presence of CuI at 50 °C in toluene. To our delight, each reaction gave only the 1,4-disubstituted triazole dendrimers **3-G22** and **3-G33** in good yields (Table 1, entries 7–8). For comparison, the reaction of **1-D2** with **2-D2** without CuI at 80 °C in toluene was conducted to provide the 1,2,3-triazole dendrimer **3-G22** with a 2.5:1 ratio of 1,4- to 1,5-isomers (Table 1, entry 6).

These observations suggested that the Cu(I) species and the steric effect of the dendron in the cycloaddition reaction between azide and alkyne is crucial to control this 1,4- versus 1,5-regioselectivity problem.

After optimization of the reaction conditions, we turned our attention toward the formation of unsymmetrical 1,2,3-triazole dendrimers. We investigated two synthetic strategies. The first one is based on the reactions between larger azido dendron **1-D3** with **2-D1** and **2-D2** (Table 1, entries 9–10). The second strategy involves the reactions between higher acetylenic dendron **2-D3** with **1-D1** and **1-**

Table 1 Synthesis of Triazole Dendrimers **3-Gmn** from Azido Dendrons **1-Dm** and Acetylenic Dendrons **2-Dn**

Entry	1-Dm	2-Dn	Additive	Reaction conditions	Product (ratio)[a]	Yield (%)[b]
1	1-D1	2-D1	none	toluene, 120 °C, 16 h	3-G11 (2:1)	90
2	1-D1	2-D1	none	toluene, 80 °C, 28 h	3-G11 (2.7:1)	98
3	1-D1	2-D1	none	toluene, 50 °C, 8 d	3-G11 (9:1)	87
4	1-D1	2-D1	CuI	toluene, 50 °C, 16 h	3-G11 (20:1)	92
5	1-D1	2-D1	CuI	toluene, r.t., 5 d	3-G11 (22:1)	93
6	1-D2	2-D2	none	toluene, 80 °C, 48 h	3-G22 (2.5:1)	81
7	1-D2	2-D2	CuI	toluene, 50 °C, 20 h	3-G22	84
8	1-D3	2-D3	CuI	toluene, 50 °C, 27 h	3-G33	81
9	1-D3	2-D1	CuI	toluene, 50 °C, 21 h	3-G31	88
10	1-D3	2-D2	CuI	toluene, 50 °C, 21 h	3-G32	89
11	1-D1	2-D3	CuI	toluene, 50 °C, 24 h	3-G13	86
12	1-D2	2-D3	CuI	toluene, 50 °C, 27 h	3-G23	84

[a] 'Ratio' represents the ratio of 1,4- to 1,5-disubstituted triazole isomers which is determined after isolation by column chromatography.
[b] Isolated yields.

D2 (Table 1, entries 11–12). The reactions of **1-D3** with **2-D1** and **2-D2** in the presence of CuI at 50 °C in toluene provided 1,4-disubstituted 1,2,3-triazole unsymmetrical dendrimers **3-G31** and **3-G32** in yields of 88% and 89%, respectively. The reactions of **1-D1** and **1-D2** with **2-D3** in the same condition gave 1,4-disubstituted 1,2,3-triazole unsymmetrical dendrimers **3-G13** and **3-G23** in yields of 87% and 84%, respectively. All compounds were confirmed by ^{1}H and ^{13}C NMR spectroscopy and FAB mass spectra. The results showed that the formation of triazole could be regarded as a new connector to construct various dendrimers from different classes of dendrons.

In summary, we demonstrated that the dimerization reactions between azido dendrons and acetylenic dendrons lead to the formation of 1,4-disubstituted triazole dendrimers in high yields. Furthermore, such reactions between dendrons of different size afford 1,4-disubstituted 1,2,3-triazole unsymmetrical dendrimers. This unique reaction may then provide an insight into designing various (un)symmetrical dendrimers such as amphiphilic dendrimers. We currently investigate the synthesis of various functional dendrimers using this strategy.

^{1}H NMR spectra were recorded on a 300 or 500 MHz NMR spectrometer using the residual proton resonance of the solvent as the internal standard. ^{13}C NMR spectra were proton decoupled and recorded on a 75 or 125 MHz NMR spectrometer using the carbon signal of the deuterated solvent as the internal standard. EI and FAB mass spectra were obtained from Korean Basic Science Institute in Daegu. Flash chromatography was performed with 37–75 μm silica gel. Analytical thin layer chromatography was performed on silica plates with F-254 indicator and the visualization was accomplished by UV lamp or using an iodine chamber. All chemicals were obtained from commercial sources and used as received, unless otherwise mentioned.

1,2,3-Triazole Dendrimers 3-Gmn via Reaction between Azido Dendrons 1-Dm and Acetylenic Dendrons 2-Dn; General Procedure

A mixture of azido dendrons **1-Dm** (0.11 mmol) and acetylenic dendrons **2-Dn** (0.10 mmol) in solvent in the absence or presence of CuI (0.10 mmol) was stirred at the designated temperature for the specified period of time (see Table 1). The progress of the reactions was monitored by TLC for the disappearance of acetylenic dendrons. The mixture was cooled to r.t. and then an aqueous workup was carried out. The desired product including the 1,5-regioisomer was isolated by column chromatography (EtOAc–*n*-hexanes system).

3-G11
^{1}H NMR (500 MHz, CDCl$_3$): δ = 3.76 (s, 6 H), 3.78 (s, 6 H), 5.30 (s, 2 H), 5.48 (s, 2 H), 6.40 (s, 2 H), 6.42 (s, 1 H), 6.44 (s, 1 H), 6.58 (s, 2 H), 7.98 (s, 1 H).

^{13}C NMR (125 MHz, CDCl$_3$): δ = 161.9, 161.3, 160.8, 140.6, 138.0, 136.1, 127.9, 106.8, 106.7, 101.2, 100.8, 67.2, 55.9, 55.8, 55.0.

MS (EI, 70 eV): *m/z* (%) = 413 [M$^+$] (33), 247 (18), 219 (97), 151 (100).

HRMS (EI): *m/z* calcd for C$_{21}$H$_{23}$N$_3$O$_6$: 413.1587; found: 413.1590.

3-G11 (1,5-Regioisomer)
^{1}H NMR (500 MHz, CDCl$_3$): δ = 3.73 (s, 6 H), 3.78 (s, 6 H), 5.24 (s, 2 H), 5.85 (s, 2 H), 6.37 (s, 1 H), 6.44 (s, 3 H), 6.50 (s, 2 H), 8.17 (s, 1 H).

^{13}C NMR (125 MHz, CDCl$_3$): δ = 161.5, 161.4, 158.5, 138.8, 137.5, 137.3, 128.0, 106.7, 106.3, 100.9, 100.8, 67.7, 55.79, 55.75, 53.8.

MS (EI, 70 eV): *m/z* (%) = 413 [M$^+$] (31), 247 (6), 191 (30), 151 (100).

HRMS (EI): *m/z* calcd for C$_{21}$H$_{23}$N$_3$O$_6$: 413.1587; found: 413.1582.

3-G22
^{1}H NMR (500 MHz, CDCl$_3$): δ = 3.78 (s, 24 H), 4.93 (s, 4 H), 4.96 (s, 4 H), 5.30 (s, 2 H), 5.47 (s, 2 H), 6.40 (m, 3 H), 6.47 (m, 2 H), 6.52 (m, 4 H), 6.55 (m, 6 H), 6.58 (m, 1 H), 6.67 (m, 2 H), 7.95 (s, 1 H).

^{13}C NMR (125 MHz, CDCl$_3$): δ = 161.1, 161.0, 160.5, 160.4, 160.0, 140.2, 139.1, 138.7, 137.7, 135.8, 127.5, 107.5, 107.4, 105.2, 102.5, 102.1, 100.1, 70.2, 70.1, 66.7, 55.4, 54.5.

MS–FAB: *m/z* = 957.9 [M$^+$], 979.8 [M$^+$ + Na].

HRMS–FAB: *m/z* [M$^+$] calcd for C$_{53}$H$_{55}$N$_3$O$_{14}$: 957.3684; found: 957.3649.

3-G22 (1,5-Regioisomer)
^{1}H NMR (500 MHz, CDCl$_3$): δ = 3.78 (s, 24 H), 4.87 (s, 4 H), 4.93 (s, 4 H), 5.20 (s, 2 H), 5.82 (s, 2 H), 6.40 (m, 4 H), 6.50 (m, 3 H), 6.52 (m, 4 H), 6.54 (m, 4 H), 6.57 (m, 3 H), 8.12 (s, 1 H).

^{13}C NMR (125 MHz, CDCl$_3$): δ = 161.4, 161.0, 160.09, 160.06, 158.0, 138.96, 138.93, 138.4, 137.1, 136.9, 127.5, 107.3, 106.9, 105.3, 102.3, 102.1, 100.0, 99.9, 70.1, 67.2, 55.4, 53.4.

MS–FAB: *m/z* = 957.9 [M$^+$], 979.8 [M$^+$ + Na].

HRMS–FAB: *m/z* [M$^+$] calcd for C$_{53}$H$_{55}$N$_3$O$_{14}$: 957.3684; found: 957.3752.

3-G33
^{1}H NMR (500 MHz, CDCl$_3$): δ = 3.76 (s, 48 H), 4.89 (s, 4 H), 4.94 (s, 20 H), 5.29 (s, 2 H), 5.41 (s, 2 H), 6.39 (m, 7 H), 6.42 (m, 3 H), 6.53 (m, 7 H), 6.55 (m, 15 H), 6.60 (m, 4 H), 6.64 (m, 6 H), 7.94 (s, 1 H).

^{13}C NMR (125 MHz, CDCl$_3$): δ = 161.1, 160.5, 160.23, 160.2, 160.1, 140.3, 139.3, 138.9, 137.9, 136.0, 127.7, 109.6, 107.5, 107.4, 106.5, 105.4, 102.5, 102.2, 101.8, 100.1, 100.0, 70.2, 66.8, 55.5, 54.5.

MS–FAB: *m/z* = 2047.9 [M$^+$].

HRMS–FAB: *m/z* [M$^+$] calcd for C$_{117}$H$_{119}$N$_3$O$_{30}$: 2045.7878; found: 2045.7822.

3-G31
^{1}H NMR (300 MHz, CDCl$_3$): δ = 3.75 (s, 6 H), 3.78 (s, 24 H), 4.91 (s, 4 H), 4.96 (s, 8 H), 5.29 (s, 2 H), 5.45 (s, 2 H), 6.40 (m, 4 H), 6.45 (m, 2 H), 6.56 (m, 14 H), 6.63 (m, 4 H), 7.97 (s, 1 H).

^{13}C NMR (75 MHz, CDCl$_3$): δ = 161.1, 161.0, 160.6, 160.5, 160.2, 140.4, 139.2, 138.8, 137.7, 135.9, 127.6, 109.5, 107.4, 106.5, 105.4, 102.5, 101.8, 100.5, 100.0, 70.2, 66.9, 55.5, 54.6.

MS–FAB: *m/z* = 1230.0 [M$^+$].

HRMS–FAB: *m/z* [M$^+$] calcd for C$_{69}$H$_{71}$N$_3$O$_{18}$: 1229.4733; found: 1229.4628.

3-G32
^{1}H NMR (500 MHz, CDCl$_3$): δ = 3.76 (s, 12 H), 3.77 (s, 24 H), 4.91 (s, 4 H), 4.93 (s, 4 H), 4.95 (s, 8 H), 5.28 (s, 2 H), 5.45 (s, 2 H), 6.39 (m, 4 H), 6.45 (m, 2 H), 6.54–6.56 (m, 18 H), 6.62 (m, 4 H), 6.65 (m, 2 H), 7.95 (s, 1 H).

^{13}C NMR (125 MHz, CDCl$_3$): δ = 161.12, 161.1, 160.6, 160.5, 160.2, 160.1, 140.3, 139.23, 139.2, 138.9, 137.8, 135.9, 127.7, 107.6, 107.4, 106.5, 105.4, 105.3, 102.5, 102.2, 101.8, 100.13, 100.1, 70.2, 66.8, 55.5, 54.6.

MS–FAB: m/z = 1502.4 [M$^+$].

HRMS–FAB: m/z [M$^+$] calcd for C$_{85}$H$_{87}$N$_3$O$_{22}$: 1501.578; found: 1501.5719.

3-G13

^{1}H NMR (300 MHz, CDCl$_3$): δ = 3.73 (s, 6 H), 3.78 (s, 24 H), 4.96 (s, 12 H), 5.29 (s, 2 H), 5.44 (s, 2 H), 6.38 (m, 2 H), 6.41 (m, 4 H), 6.54–6.57 (m, 12 H), 6.66 (m, 6 H), 7.96 (s, 1 H).

^{13}C NMR (75 MHz, CDCl$_3$): δ = 161.6, 161.1, 160.5, 160.2, 160.1, 140.3, 139.3, 137.8, 135.9, 127.7, 107.6, 106.5, 106.4, 105.4, 102.2, 101.8, 100.8, 100.1, 70.2, 70.14, 66.8, 55.6, 55.5, 54.6.

MS–FAB: m/z = 1229.9 [M$^+$].

HRMS–FAB: m/z [M$^+$] calcd for C$_{69}$H$_{71}$N$_3$O$_{18}$: 1229.4733; found: 1229.4871.

3-G23

^{1}H NMR (500 MHz, CDCl$_3$): δ = 3.77 (s, 36 H), 4.90 (s, 4 H), 4.95 (s, 12 H), 5.30 (s, 2 H), 5.42 (s, 2 H), 6.39 (m, 6 H), 6.44 (m, 2 H), 6.51 (m, 4 H), 6.54–6.56 (m, 12 H), 6.65 (m, 6 H), 7.93 (s, 1 H).

^{13}C NMR (125 MHz, CDCl$_3$): δ = 161.22, 161.2, 160.64, 160.57, 160.3, 160.2, 140.4, 139.4, 139.3, 138.9, 137.9, 136.0, 127.7, 107.7, 107.5, 106.5, 105.5, 105.4, 102.6, 102.3, 101.9, 100.21, 100.17, 70.32, 70.26, 70.2, 66.9, 55.6, 54.6.

MS–FAB: m/z = 1502.4 [M$^+$].

HRMS–FAB: m/z [M$^+$] calcd for C$_{85}$H$_{87}$N$_3$O$_{22}$: 1501.5781; found: 1501.5897.

Acknowledgment

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A Study of the Scope and Regioselectivity of the Ruthenium-Catalyzed [3 + 2]-Cycloaddition of Azides with Internal Alkynes

Max M. Majireck and Steven M. Weinreb*

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

smw@chem.psu.edu
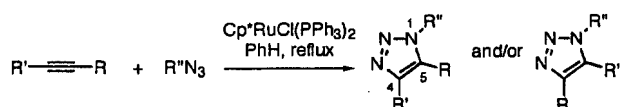
[3 + 2]-Cycloadditions of alkyl azides with various unsymmetrical internal alkynes in the presence of Cp*RuCl(PPh3)2 as catalyst in refluxing benzene have been examined, leading to 1,4,5-trisubstituted-1,2,3-triazoles. Whereas alkyl phenyl and dialkyl acetylenes undergo cycloadditions to afford mixtures of regioisomeric 1,2,3-triazoles, acyl-substituted internal alkynes react with complete regioselectivity. In addition, propargyl alcohols and propargyl amines were found to react with azides to afford single regioisomeric products.

1,2,3-Triazoles are nitrogen heteroarenes which have found a range of important applications in the pharmaceutical and agricultural industries.[1] The most widely used method for synthesis of 1,2,3-triazoles has involved the thermal 1,3-dipolar cycloaddition of organic azides with alkynes pioneered by Huisgen.[2] However, there are major problems commonly associated with this methodology, including the need for long reaction times and high temperatures, as well as the formation of regioisomeric mixtures of products when using unsymmetrical alkynes. It was recently reported that it is possible to impart some regioselectivity into these thermal cycloadditions by utilizing sterically or electronically biased alkynes.[3] As part of the work by the Sharpless group on so-called "click" reactions,[4] whereby heteroatom links between molecules can be generated under mild conditions, it was found that cycloadditions of terminal alkynes with alkyl azides catalyzed by Cu(I) can be conducted at room temperature and are highly regioselective.[5,6] This methodology was also independently discovered by Meldal et al. at about the same time.[7] Thus, cycloadditions of alkynes 1 with azides 2 under conditions such as those shown in Scheme 1 lead exclusively to 4-substituted-1,2,3-triazoles 3 in high yields. This type of copper catalysis, however, does not promote the cycloadditions of internal alkynes. Mechanistic studies have demonstrated that these reactions involve terminal copper acetylides and proceed via a stepwise non-concerted process.[5b]

More recently, it was discovered that the reaction of terminal alkynes with alkyl azides is catalyzed by Cp*RuCl(PPh3)2 in refluxing benzene to afford exclusively the 1,5-disubstituted-1,2,3-triazoles 4 (Scheme 1).[8] In contrast to the click reactions promoted by copper, the ruthenium complex was also reported to catalyze the cycloaddition reaction of internal alkynes, although only a single case which involved a symmetrical system was described (eq 1).
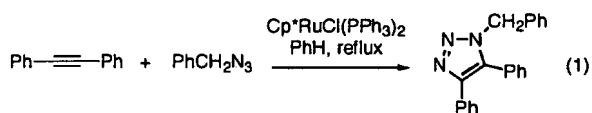
It was suggested that this transformation probably occurs by initial coordination of the alkyne and azide with catalyst 5 to afford intermediate 6, which then undergoes cyclotrimerization to afford metallacycle 7 or 8 (Scheme 2). The formation of the former metallacycle 7 seems most likely due to unfavorable steric interactions in isomer 8. Reductive elimination of this intermediate then leads to the 1,2,3-triazole and regenerates the catalyst. No rationale was presented, however, for the preference for the observed formation of the 1,5-disubstituted triazole system 4 when using terminal alkynes. As part of our recent interest in 1,2,3-triazoles,[9] we decided to explore the generality and scope of the Ru-catalyzed cycloadditions of alkyl azides with internal alkynes.

Initial experiments were conducted with benzyl azide and commercially available alkynes to form 1,4,5-trisubstituted-1,2,3-triazoles (A and/or B) as outlined in Table 1. The reactions were all run with 10 mol % of ruthenium catalyst 5 in refluxing benzene. In the majority of cases, the cycloadditions proceeded to completion within 2.5 h using ~1.5 equiv of acetylene relative

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SCHEME 1
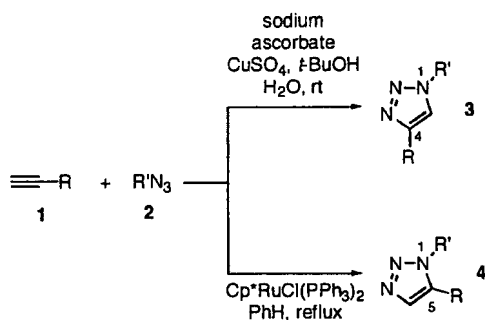
SCHEME 2
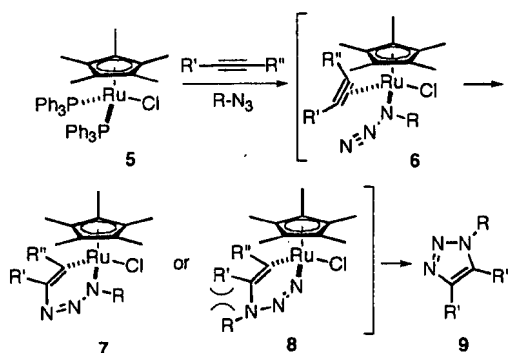
to azide, giving good yields of products. In reactions which were slower, however, the amount of alkyne was increased, as was the reaction time. In cases where regioisomers were formed, NMR integration of the crude reaction mixture prior to chromatographic separation was utilized to determine product ratios. The regiochemistry of the purified products was then established by ^{1}H NMR NOE experiments. In the cases of phenyl alkyl acetylenes (entries 1 and 2), moderate regioselectivity was observed in favor of isomers B. With unsymmetrical methyl propyl acetylene (entry 7), the A/B ratio was about 1/2. In the case of hindered methyl t-butyl acetylene, the cycloaddition proved to be quite slow, and although only one regioisomeric triazole B was detected, the yield was low.

A few unsymmetrical disubstituted alkynes which bear a carbonyl group were also investigated (entries 3, 4, 11, and 12), and in all cases, the cycloadditions were totally regioselective, affording only triazole isomers A. It should be noted that thermal (uncatalyzed) cycloadditions of electron-deficient alkynes with azides usually produce mixtures of regioisomeric products,[2b] although isomers such as A usually predominate, presumably for polarity reasons. It has recently been found that, if these thermal reactions are conducted in water, isomers of type A are produced cleanly with both terminal and internal alkynes.[3c]

We were surprised to find that propargylic alcohols undergo Ru-catalyzed cycloadditions to afford exclusively 1,2,3-triazoles of type B (entries 5 and 14). On the other hand, in the case of a homopropargyl alcohol (entry 10), the regioselectivity is low and is similar to that found with a simple dialkyl acetylene (cf. entry 7). Moreover, the alkynyl acetal in entry 6 showed no regioselectivity in the cycloaddition, which would seem to preclude any type of heteroatom—metal coordination.[10] The same high regioselectivity in favor of triazole B was observed

(10) See for example: Na, Y.; Chang, S. Org. Lett. 2000, 2, 1887.

with a propargyl amine (entry 13). It should also be noted that attempted cycloaddition of benzyl azide with phenyl trimethylsilyl acetylene provided a complex mixture of products.

The cycloaddition of benzhydryl azide[11] with methyl phenyl acetylene was also examined. This secondary azide reacts more slowly than does benzyl azide, but affords the same ratio of adducts A/B (entry 15). In the case of highly hindered, tertiary adamantyl azide, the reaction is extremely slow and provides a single product of type B, but only in very low yield (entry 16).

We have shown that the reaction of internal alkynes with azides catalyzed by Cp*RuCl(PPh₃)₂ (5) is a general process. These cycloadditions proceed under mild conditions, affording 1,4,5-trisubstituted-1,2,3-triazoles in good yields. Depending upon the alkyne substitution pattern, the reactions can be highly regioselective. At present, however, we are unable to offer a compelling mechanistic rationale for these regiochemical results.

Experimental Section

General Procedure for Ru-Catalyzed Cycloadditions. A mixture of azide (1.0 equiv, 0.5 mmol), alkyne (1.2—5.0 equiv), Cp*RuCl(PPh₃)₂ (0.1 equiv, 0.05 mmol), and 2.5 mL of anhydrous benzene was refluxed at 80 °C for 2.5—40 h. The progress of the reaction was monitored by TLC. The mixture was then cooled and evaporated under reduced pressure. The product was purified by flash column chromatography using a mixture of ethyl acetate and hexanes. Ratios of regioisomers (see Table 1) were determined on the crude reaction mixture by ^{1}H NMR integration prior to chromatography. Numbers of structures below refer to Table 1. Compounds 1A,[12] 1B,[13] 3A,[14] 4A,[13,15] and 9B[12] have been previously prepared.

1-Benzyl-4-methyl-5-phenyl-1H-[1,2,3]triazole (entry 1A) and 1-Benzyl-5-methyl-4-phenyl-1H-[1,2,3]triazole (entry 1B). Benzyl azide (67 mg, 0.50 mmol), prop-1-ynylbenzene (125 mg, 1.08 mmol), and Cp*RuCl(PPh₃)₂ (40 mg, 0.05 mmol). The products were obtained as a yellow oil (42 mg of 1A) and a white solid (78 mg of 1B) in a total yield of 95%. 1A: ^{1}H NMR (300 MHz, CDCl₃) δ 7.46—7.44 (m, 3H), 7.30—7.25 (m, 3H), 7.20—7.15 (m, 2H), 7.07—7.03 (m, 2H), 5.45 (s, 2H), 2.34 (s, 3H); ^{13}C NMR (75 MHz, CDCl₃) δ 142.1, 135.9, 135.1, 129.9, 129.7, 129.3, 129.1, 128.5, 127.7, 52.5, 11.1; LRMS-ES+ m/z (relative intensity) 250 (MH⁺, 65); HRMS-ES+ (C₁₆H₁₅N₃) calcd 250.1344 (MH⁺), found 250.1349. 1B: ^{1}H NMR (300 MHz, CDCl₃) δ 7.74—7.71 (m, 2H), 7.49—7.21 (m, 8H), 5.57 (s, 2H), 2.36 (s, 3H); ^{13}C NMR (75 MHz, CDCl₃) δ 145.4, 135.3, 132.0, 129.6, 129.5, 129.1, 128.7, 128.1, 127.59, 127.57, 52.5, 9.6; LRMS-ES+ m/z (relative intensity) 250 (MH⁺, 65); HRMS-ES+ (C₁₆H₁₅N₃) calcd 250.1344 (MH⁺), found 250.1349.

1-Benzyl-5-phenyl-4-propyl-1H-[1,2,3]triazole (entry 2A) and 1-Benzyl-4-phenyl-5-propyl-1H-[1,2,3]triazole (entry 2B). Benzyl azide (67 mg, 0.50 mmol), pent-1-ynylbenzene (110 mg, 0.76 mmol), and Cp*RuCl(PPh₃)₂ (40 mg, 0.05 mmol). Both products were obtained as yellow oils (14 mg of 2A; 96 mg of 2B) in a total yield of 80%. 2A: ^{1}H NMR (300 MHz, CDCl₃) δ 7.44—7.01 (m, 10H), 5.41 (s, 2H), 2.61 (t, J = 7.5 Hz, 2H), 1.67 (sextet, J = 7.6 Hz, 2H), 0.87 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, CDCl₃) δ 146.3, 136.1, 130.2, 129.6, 129.3, 129.2, 129.1, 128.4, 128.0, 127.8, 52.4, 27.5, 23.3, 14.3; LRMS-ES+ m/z (relative intensity)

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TABLE 1. Preparation of 1,4,5-Trisubstituted-1,2,3-triazoles via Ru-Catalyzed Cycloadditions of Azides with Internal Alkynes

$$R_1\text{—}\!\equiv\!\text{—}R_2 + R_3\text{—}N\!=\!\overset{\oplus}{N}\!=\!\overset{\ominus}{N}: \xrightarrow[\text{PhH, 80 °C}]{10 \text{ mol } \% \text{ Cp*RuCl(PPh}_3)_2} \quad \underset{\mathbf{A}}{R_1\text{—triazole}} + \underset{\mathbf{B}}{R_2\text{—triazole}}$$
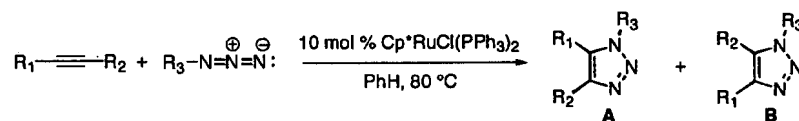
entry	R_1	R_2	R_3	equiv of alkyne	time (h)	% yield	A:B ratio
1	Ph	Me	CH$_2$Ph	2.0	2.5	95	38:62
2	Ph	Pr	CH$_2$Ph	1.5	2.5	80	13:87
3	Ph	CO$_2$Et	CH$_2$Ph	2.0	2.5	85	100:0
4	Ph	COMe	CH$_2$Ph	1.5	2.5	100	100:0
5	Ph	CH$_2$OH	CH$_2$Ph	1.5	5.0	70	0:100
6	Ph	CH(OEt)$_2$	CH$_2$Ph	1.5	2.5	75	50:50
7	Me	Pr	CH$_2$Ph	3.0	16	75	32:68
8	Et	Et	CH$_2$Ph	2.0	6.0	85	N/A
9	Me	*t*-Bu	CH$_2$Ph	5.0	36	15	0:100
10	Me	CH$_2$CH$_2$OH	CH$_2$Ph	1.5	8.0	90	23:77
11	Bu	CO$_2$Me	CH$_2$Ph	1.2	2.5	90	100:0
12	Et	COMe	CH$_2$Ph	1.5	2.5	90	100:0
13	Me	CH$_2$NEt$_2$	CH$_2$Ph	1.5	2.5	70	0:100
14	Et	CMe$_2$OH	CH$_2$Ph	1.5	5.0	80	0:100
15	Ph	Me	CHPh$_2$	3.0	20	65	33:67
16	Ph	Me	1-adamantyl	5.0	40	10	0:100

278 (MH$^+$, 100); HRMS-ES+ (C$_{18}$H$_{20}$N$_3$) calcd 278.1657 (MH$^+$), found 278.1638. **2B**: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.74–7.70 (m, 2H), 7.48–7.21 (m, 8H), 5.58 (s, 2H), 2.72 (t, J = 6.3 Hz, 2H), 1.43 (sextet, J = 8.1 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 145.2, 135.7, 133.9. 132.2, 129.4, 129.1, 128.7, 128.1, 127.51, 127.47, 52.5, 25.6, 22.3, 14.4; LRMS-ES+ *m/z* (relative intensity) 278 (MH$^+$, 100); HRMS-ES+ (C$_{18}$H$_{20}$N$_3$) calcd 278.1657 (MH$^+$), found 278.1638.

1-Benzyl-5-phenyl-1H-[1,2,3]triazole-4-carboxylic Acid Ethyl Ester (entry 3A). Benzyl azide (67 mg, 0.50 mmol), phenylpropynoic acid ethyl ester (165 mg, 0.95 mmol), and Cp*RuCl(PPh$_3$)$_2$ (40 mg, 0.05 mmol). The product was obtained as an off-white solid (131 mg) in 85% yield: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.50–7.41 (m, 3H), 7.28–7.18 (m, 5H), 7.01–6.98 (m, 2H), 5.44 (s, 2H), 4.29 (q, J = 7.2 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 161.3, 141.7, 137.5, 135.0, 130.5, 130.2, 129.2, 128.9, 128.8, 127.9, 126.4, 61.4, 52.6, 14.5; LRMS-ES+ *m/z* (relative intensity) 308 (MH$^+$, 100); HRMS-ES+ (C$_{18}$H$_{17}$N$_3$O$_2$) calcd 308.1399 (MH$^+$), found 308.1377.

1-(1-Benzyl-5-phenyl-1H-[1,2,3]triazol-4-yl)-ethanone (entry 4A). Benzyl azide (67 mg, 0.50 mmol), 4-phenylbut-3-yn-2-one (110 mg, 0.76 mmol), and Cp*RuCl(PPh$_3$)$_2$ (40 mg, 0.05 mmol). The product was obtained as a yellow oil (140 mg) in 100% yield: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.51–7.43 (m, 3H), 7.29–7.25 (m, 3H), 7.23–7.21 (m, 2H), 7.04–7.02 (m, 2H), 5.43 (s, 2H), 2.69 (s, 3H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 193.1, 144.2, 140.0, 135.1, 131.0, 130.1, 129.3, 129.1, 128.9, 128.0, 126.4, 52.4, 28.5; LRMS-ES+ *m/z* (relative intensity) 300 (M + Na$^+$, 95); HRMS-ES+ (C$_{17}$H$_{16}$N$_3$O) calcd 278.1293 (MH$^+$), found 278.1312.

(3-Benzyl-5-phenyl-3H-[1,2,3]triazol-4-yl)methanol (entry 5B). Benzyl azide (67 mg, 0.50 mmol), 3-phenylprop-2-yn-1-ol (100 mg, 0.75 mmol), and Cp*RuCl(PPh$_3$)$_2$ (40 mg, 0.05 mmol). The product was obtained as an off-white solid (93 mg) in 70% yield: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.66–7.63 (m, 2H), 7.39–7.23 (m, 8H), 5.62 (s, 2H), 4.69 (s, 2H), 3.83 (s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 146.6, 135.4, 132.6, 130.9, 129.4, 129.2, 128.8, 128.7, 128.1, 128.0, 52.9, 52.7; LRMS-ES+ *m/z* (relative intensity) 266 (MH$^+$, 100); HRMS-ES+ (C$_{16}$H$_{16}$N$_3$O) calcd 266.1293 (MH$^+$), found 266.1273.

1-Benzyl-4-diethoxymethyl-5-phenyl-1H-[1,2,3]triazole (entry 6A) and 1-Benzyl-5-diethoxymethyl-4-phenyl-1H-[1,2,3]triazole (entry 6B). Benzyl azide (67 mg, 0.50 mmol), (3,3-diethoxyprop-1-ynyl)benzene (155 mg, 0.76 mmol), and Cp*RuCl(PPh$_3$)$_2$ (40 mg,

0.05 mmol). The products were obtained as a yellow oil (45 mg of **6A**) and a clear oil (80 mg of **6B**) in a total yield of 75%. **6A**: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.42–7.40 (m, 3H), 7.26–7.20 (m, 5H), 7.04–7.01 (m, 2H), 5.60 (s, 1H), 5.43 (s, 2H), 3.71–3.64 (m, 2H), 3.59–3.52 (m, 2H), 1.12 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 143.8, 135.7, 130.4, 129.8, 129.1, 128.9, 128.5, 127.8, 127.2, 96.8, 61.9, 52.3, 15.4; LRMS-ES+ *m/z* (relative intensity) 338 (MH$^+$, 100); HRMS-ES+ (C$_{20}$H$_{24}$N$_3$O$_2$) calcd 338.1869 (MH$^+$), found 338.1855. **6B**: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.73–7.70 (m, 2H), 7.50–7.33 (m, 8H), 5.82 (s, 2H), 5.64 (s, 1H), 3.66–3.56 (m, 2H), 3.43–3.35 (m, 2H), 1.09 (t, J = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 146.6, 136.5, 131.2, 131.1, 128.9, 128.8, 128.6, 128.3, 128.2, 96.2, 63.6, 53.7, 15.2; LRMS-ES+ *m/z* (relative intensity) 338 (MH$^+$, 100); HRMS-ES+ (C$_{20}$H$_{24}$N$_3$O$_2$) calcd 338.1869 (MH$^+$), found 338.1855.

1-Benzyl-5-methyl-4-propyl-1H-[1,2,3]triazole (entry 7A) and 1-Benzyl-4-methyl-5-propyl-1H-[1,2,3]triazole (entry 7B). Benzyl azide (67 mg, 0.50 mmol), hex-2-yne (125 mg, 1.5 mmol), and Cp*RuCl(PPh$_3$)$_2$ (40 mg, 0.05 mmol). Both products were obtained as clear oils (25 mg of **7A**; 56 mg of **7B**) in 75% total yield. **7A**: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.35–7.32 (m, 3H), 7.17–7.15 (m, 2H), 5.48 (s, 2H), 2.60 (t, J = 7.4 Hz, 2H), 2.09 (s, 3H), 1.70 (q, J = 7.5 Hz, 2H), 0.95 (t, J = 7.4 Hz, 3H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 146.0, 135.5, 129.3, 128.6, 127.50, 127.47, 52.3, 27.5, 23.2, 14.2, 8.3; LRMS-ES+ *m/z* (relative intensity) 216 (MH$^+$, 100); HRMS-ES+ (C$_{13}$H$_{17}$N$_3$) calcd 216.1501 (MH$^+$), found 216.1495. **7B**: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.33–7.29 (m, 3H), 7.16–7.14 (m, 2H), 5.47 (s, 2H), 2.46 (t, J = 7.6 Hz, 2H), 2.28 (s, 3H), 1.36 (q, J = 7.6 Hz, 2H), 0.84 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 141.7, 135.9, 133.6, 129.3, 128.6, 127.4, 52.3, 24.9, 22.1, 14.1, 11.0; LRMS-ES+ *m/z* (relative intensity) 216 (MH$^+$, 100); HRMS-ES+ (C$_{13}$H$_{17}$N$_3$) calcd 216.1501 (MH$^+$), found 216.1495.

1-Benzyl-4,5-diethyl-1H-[1,2,3]triazole (entry 8). Benzyl azide (67 mg, 0.50 mmol), hex-3-yne (82 mg, 1.0 mmol), and Cp*RuCl(PPh$_3$)$_2$ (40 mg, 0.05 mmol). The product was obtained as a yellow oil (90 mg) in 85% yield: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.30–7.27 (m, 3H), 7.15–7.11 (m, 2H), 5.45 (s, 2H), 2.62 (q, J = 7.6 Hz, 2H), 2.51 (q, J = 7.7 Hz, 2H), 1.26 (t, J = 7.6 Hz, 3H), 0.94 (t, J = 7.6 Hz, 3H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 146.7, 135.9, 134.4, 129.3, 128.5, 127.4, 52.2, 18.9, 16.3, 14.6, 13.7; LRMS-ES+ *m/z* (relative intensity) 216 (MH$^+$, 100); HRMS-ES+ (C$_{13}$H$_{18}$N$_3$) calcd 216.1501 (MH$^+$), found 216.1500.

1-Benzyl-5-*tert*-butyl-4-methyl-1*H*-[1,2,3]triazole (entry 9B). Benzyl azide (67 mg, 0.50 mmol), 4,4-dimethylpent-2-yne (245 mg, 2.5 mmol), and Cp*RuCl(PPh₃)₂ (40 mg, 0.05 mmol). The product was obtained as a clear oil (17 mg) in 15% yield: ¹H NMR (400 MHz, CDCl₃) δ 7.32−7.27 (m, 3H), 6.99−6.97 (m, 2H), 5.73 (s, 2H), 2.50 (s, 3H), 1.32 (s, 9H); ¹³C NMR (75 MHz, CDCl₃) δ 140.5, 140.1, 137.2, 129.2, 128.1, 126.5, 54.2, 31.9, 30.9, 15.0; LRMS-ES+ *m/z* (relative intensity) 230 (MH⁺, 55); HRMS-ES+ (C₁₄H₂₀N₃) calcd 230.1657 (MH⁺), found 230.1647.

2-(3-Benzyl-5-methyl-3*H*-[1,2,3]triazol-4-yl)-ethanol (entry 10A) and 2-(1-Benzyl-5-methyl-1*H*-[1,2,3]triazol-4-yl)-ethanol (entry 10B). Benzyl azide (67 mg, 0.50 mmol), pent-3-yn-1-ol (130 mg, 1.5 mmol), and Cp*RuCl(PPh₃)₂ (40 mg, 0.05 mmol). The products were obtained as an inseparable mixture (99 mg) in 90% overall yield. **10A + 10B:** ¹H NMR (400 MHz, CDCl₃) δ 7.33−7.27 (m, 3H *major* + 3H *minor*), 7.14−7.12 (m, 2H *major* + 2H *minor*), 5.55 (s, 2H *major*), 5.44 (s, 2H *minor*), 3.92 (t, *J* = 5.9 Hz, 2H *minor*), 3.72 (t, *J* = 6.1 Hz, 2H *major*), 2.81 (t, *J* = 5.9 Hz, 2H *minor*), 2.72 (t, *J* = 6.1 Hz, 2H *major*), 2.20 (s, 3H *major*), 2.04 (s, 3H *minor*); ¹³C NMR (75 MHz, CDCl₃) major peaks δ 142.2, 135.5, 134.8, 132.2, 129.4, 129.3, 129.1, 128.8, 128.7, 127.8, 127.6, 65.0, 60.7, 52.7, 52.2, 30.1, 26.5, 14.1, 10.7; LRMS-ES+ *m/z* (relative intensity) 218 (MH⁺, 100); HRMS-ES+ (C₁₂H₁₅N₃-ONa) calcd 240.1113 (M + Na⁺), found 240.1095.

1-Benzyl-5-butyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Methyl Ester (entry 11A). Benzyl azide (67 mg, 0.50 mmol), hept-2-ynoic acid methyl ester (80 mg, 0.61 mmol), and Cp*RuCl(PPh₃)₂ (40 mg, 0.05 mmol). The product was obtained as a yellow oil (114 mg) in 90% yield: ¹H NMR (400 MHz, CDCl₃) δ 7.34−7.32 (m, 3H), 7.17−7.15 (m, 2H), 5.53 (s, 2H), 3.92 (s, 3H), 2.85 (t, *J* = 8.0 Hz, 2H), 1.31−1.22 (m, 6H), 0.82 (t, *J* = 6.8 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 162.4, 143.3, 136.9, 134.9, 129.5, 129.0, 127.6, 52.5, 52.3, 30.7, 23.4, 22.9, 14.0; LRMS-ES+ *m/z* (relative intensity) 296 (M + Na⁺, 100); HRMS-ES+ (C₁₅H₁₉N₃O₂) calcd 296.1375 (M + Na⁺), found 296.1377.

1-(1-Benzyl-5-ethyl-1*H*-[1,2,3]triazol-4-yl)-ethanone (entry 12A). Benzyl azide (67 mg, 0.50 mmol), hex-3-yn-2-one (73 mg, 0.75 mmol), and Cp*RuCl(PPh₃)₂ (40 mg, 0.05 mmol). The product was obtained as a yellow oil (105 mg) in 90% yield: ¹H NMR (400 MHz, CDCl₃) δ 7.35−7.28 (m, 4H), 7.18−7.16 (m, 2H), 5.51 (s, 2H), 2.89 (q, *J* = 7.5 Hz, 2H), 2.67 (s, 3H), 0.95 (t, *J* = 7.1 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 194.4, 143.9, 142.7, 134.9, 129.5, 129.0, 127.6, 52.0, 28.1, 17.3, 12.6; LRMS-ES+ *m/z* (relative intensity) 252 (M + Na⁺, 100); HRMS-ES+ (C₁₃H₁₅N₃ONa) calcd 252.1113 (MH⁺), found 252.1113.

(3-Benzyl-5-methyl-3*H*-[1,2,3]triazol-4-ylmethyl)diethylamine (entry 13B). Benzyl azide (67 mg, 0.50 mmol), but-2-ynyldiethylamine (85 mg, 0.75 mmol), and Cp*RuCl(PPh₃)₂ (40 mg, 0.05 mmol). The product was obtained as a yellow oil (90 mg) in 70% yield: ¹H NMR (400 MHz, CDCl₃) δ 7.32−7.25 (m, 3H), 7.17−7.15 (m, 2H), 5.85 (s, 2H), 3.36 (s, 2H), 2.42 (q, *J* = 7.1 Hz, 4H), 2.29 (s, 3H), 0.95 (t, *J* = 7.1 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 143.1, 136.2, 130.9, 129.1, 128.3, 127.6, 52.3, 46.7, 45.9, 11.7, 10.7; LRMS-ES+ *m/z* (relative intensity) 259 (MH⁺, 100); HRMS-ES+ (C₁₅H₂₃N₄) calcd 259.1923 (MH⁺), found 259.1907.

2-(3-Benzyl-5-ethyl-3*H*-[1,2,3]triazol-4-yl)-propan-2-ol (entry 14B). Benzyl azide (67 mg, 0.50 mmol), 2-methylhex-3-yn-2-ol (83 mg, 0.74 mmol), and Cp*RuCl(PPh₃)₂ (40 mg, 0.05 mmol). The product was obtained as an off-white solid (96 mg) in 80% yield: ¹H NMR (300 MHz, CDCl₃) δ 7.29−7.23 (m, 3H), 7.14−7.12 (m, 2H), 5.83 (s, 2H), 3.48 (s, 1H), 2.71 (q, *J* = 7.5 Hz, 2H), 1.51 (s, 6H), 1.26 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 144.6, 138.1, 137.7, 129.0, 128.0, 127.5, 69.8, 54.2, 31.2, 20.7, 14.8; LRMS-ES+ *m/z* (relative intensity) 246 (MH⁺, 100); HRMS-ES+ (C₁₄H₂₀N₃O) calcd 246.1606 (MH⁺), found 246.1604.

1-Benzhydryl-4-methyl-5-phenyl-1*H*-[1,2,3]triazole (entry 15A) and 1-Benzhydryl-5-methyl-4-phenyl-1*H*-[1,2,3]triazole (entry 15B). Benzhydryl azide[11] (105 mg, 0.50 mmol), prop-1-ynylbenzene (175 mg, 1.5 mmol), and Cp*RuCl(PPh₃)₂ (40 mg, 0.05 mmol). Both products were obtained as yellow solids (30 mg of 15A; 70 mg of 15B) in 65% overall yield. **15A:** ¹H NMR (300 MHz, CDCl₃) δ 7.50−7.48 (m, 3H), 7.34−7.31 (m, 6H), 7.24−7.19 (m, 6H), 6.55 (s, 1H), 2.34 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 141.3, 139.4, 135.7, 130.2, 129.8, 129.5, 129.0, 128.8, 128.5, 128.1, 66.2, 11.1; LRMS-ES+ *m/z* (relative intensity) 326 (MH⁺, 100); HRMS-ES+ (C₂₂H₂₀N₃) calcd 326.1657 (MH⁺), found 326.1666. **15B:** ¹H NMR (400 MHz, CDCl₃) δ 7.74−7.72 (m, 2H), 7.48−7.45 (m, 2H), 7.42−7.34 (m, 7H), 7.29−7.25 (m, 4H), 6.85 (s, 1H), 2.40 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 145.2, 138.2, 132.0, 130.0, 129.2, 129.0, 128.9, 128.7, 128.1, 127.8, 66.8, 10.0; LRMS-ES+ *m/z* (relative intensity) 326 (MH⁺, 100); HRMS-ES+ (C₂₂H₂₀N₃) calcd 326.1657 (MH⁺), found 326.1666.

1-Adamantan-1-yl-5-methyl-4-phenyl-1*H*-[1,2,3]triazole (entry 16B). 1-Azidoadamantane (95 mg, 0.50 mmol), prop-1-ynylbenzene (305 mg, 2.63 mmol), and Cp*RuCl(PPh₃)₂ (40 mg, 0.05 mmol). The product was obtained as a yellow oil (13 mg) in 10% yield: ¹H NMR (400 MHz, CDCl₃) δ 7.64−7.61 (m, 2H), 7.48−7.44 (m, 2H), 7.38−7.37 (m, 1H), 2.62 (s, 3H), 2.44 (d, *J* = 3.0 Hz, 6H), 2.30 (m, 3H), 1.83 (t, *J* = 3.1 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 146.8, 132.3, 128.9, 128.5, 127.9, 62.3, 42.0, 36.4, 30.1, 12.4; LRMS-ES+ *m/z* (relative intensity) 293 (M⁺, 100); HRMS-ES+ (C₁₉H₂₃N₃) calcd 293.1892 (MH⁺), found 293.1888.

Note Added after ASAP Publication. Table 1 had an incorrect ratio in the version published October 5, 2006; the correct version was published October 5, 2006.

Supporting Information Available: Copies of proton and carbon NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JOC Article

A Potentially Valuable Advance in the Synthesis of Carbohydrate-Based Anticancer Vaccines through Extended Cycloaddition Chemistry

Qian Wan,[†] Jiehao Chen,[†] Gong Chen,[†] and Samuel J. Danishefsky*,[†,‡]

Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021, and Department of Chemistry, Columbia University, Havemeyer Hall, 3000 Broadway, New York, New York 10027

s-danishefsky@ski.mskcc.org

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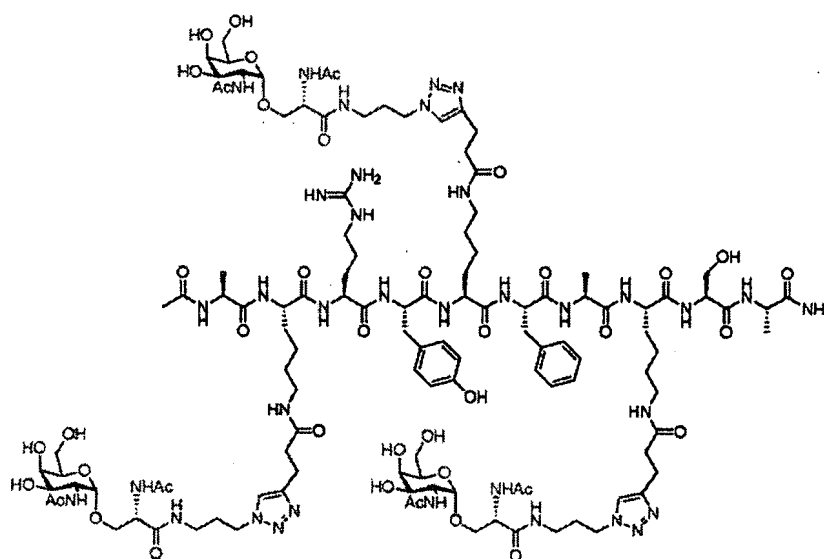
An advance in the long-term problem of joining complex oligosaccharides to polypeptides and even proteins is described herein. The key method involves equipping the oligosaccharide sector with an azide and the polypeptide with pendant alkynyl functionality. The two sectors are joined through a "click-like" cycloaddition. The method encompasses oligosaccharide constructs with several azide linkages which undergo concurrent cycloaddition to peptide-based acetylenes. This technology could well prove to be useful in the construction of fully synthetic vaccines.

Introduction

Our laboratory has been pursuing the development of clinically useful, carbohydrate-based antitumor vaccines based on extensive findings that cancer cells typically display aberrant levels and patterns of cell surface glycosylation.[1] Conceivably, by exposing a host system to molecules containing cancer-associated cell surface carbohydrate antigens, it could be possible to evoke an exploitable antitumor immune response. It has been found that one of the most effective ways to induce antibodies against a carbohydrate antigen is through conjugation of the antigen to large biomolecules. To date, we have focused on the particular carrier protein, keyhole limpet hemocyanin (KLH).[2] Our preoccupation with KLH did not arise from a confidence that it is optimally structured to serve as the ideal antigen carrier. However, not withstanding issues of homogeneity criteria, lack of structural definition, solubility, and incorporation of trace
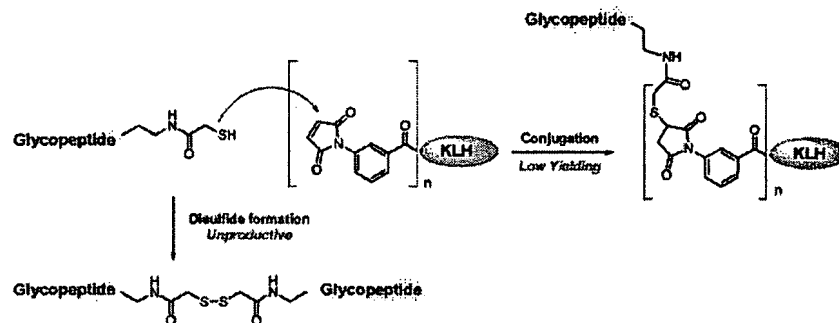
FIGURE 1. Current conjugation protocol.

metals, KLH is, at this stage of development, the most broadly employed carrier in preclinical and clinical evaluation.

Over the course of many years, our laboratory has registered a series of advances in carbohydrate and glycopeptide assembly techniques. These have enabled the de novo synthesis and Phase I clinical evaluation of increasingly sophisticated carbohydrate-based antitumor vaccine constructs. Several of our monomeric and clustered carbohydrate antigen-KLH constructs have been evaluated in preclinical and early clinical settings. One particularly promising candidate, Globo-H-KLH, is scheduled for advancement to Phase II/III clinical trials for breast cancer.[3]

More recently, we have focused our efforts on the synthesis of more complex, multiantigenic vaccine constructs, designed to be responsive to the heterogeneity of tumor cell surface glycosylation.[4] We reasoned that, by combining several different tumor-associated carbohydrate antigens on a single molecule, it should be possible to target and eliminate a greater percentage of malignantly transformed cells. Accordingly, we prepared a number of unimolecular, multiantigenic vaccine constructs, each composed of a polypeptide backbone displaying anywhere from three to six different tumor-associated carbohydrate antigens.[5] These multivalent constructs have been shown, in preclinical settings, to effectively induce antibodies against the component antigens at levels roughly equivalent to those achieved with the corresponding monovalent constructs.

The syntheses of these constructs commence with the preparation of the component glycosylamino acids. These are then iteratively coupled to form the fully glycosylated polypeptide backbone. The glycopeptide is conjugated to the carrier, for the moment, KLH (Figure 1). Typically, a sulfhydryl handle installed at the N-terminus participates in a Michael-type addition to a maleimide functionality on the surface of the modified KLH. This conjugation step had occurred, albeit in disappointingly low yields, possibly as a result of a nonproductive disulfide dimerization of the glycopeptide. Given the complexity of building the multiantigenic glycopeptide fragments, the substantial loss of material associated with this conjugation protocol is particularly egregious.

In targeting increasingly complex glycopeptide fragments for conjugation to various protein-based carriers, we sought a protocol which would allow for the reliable functionalization of each component in advance of the conjugation event. Furthermore, the chemistry used in the conjugation must be compatible with the chemistry of the components. Finally, the conjugation should proceed with a fairly rapid reaction rate, to allow for the introduction of a high percentage of vaccine molecules to the surface of the carrier protein.[6]

With these considerations in mind, we were drawn to the Cu[I]-catalyzed version of the Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes, developed independently by the Sharpless and Meldal groups.[7] Since the pioneering investigations at Scripps, this commendably straightforward chemistry (which can be conducted in aqueous media!) has been widely applied as a powerful tool for the selective modifications of enzymes,[8] viruses,[9] and cells.[10] Notably, as practiced by Sharpless and associates, the cycloaddition reaction is highly chemoselective and tolerates a variety of functionality. Furthermore, such reactions can be conducted under aqueous conditions, such as would be necessary to preserve the nature of the carrier protein.[11] The installation of the requisite azido and acetylenic functional handles on glycopeptides and carrier protein seemed likely to be a straightforward matter. Indeed, herein, we describe the development of an efficient 1,3-dipolar cycloaddition protocol which results in the merger of the carbohydrate and polypeptide domains. This logic is likely to prove applicable

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FIGURE 2. Strategy based on azide–acetylenic cycloaddition.

SCHEME 1. Preparation of 7, 8, and 12[a]
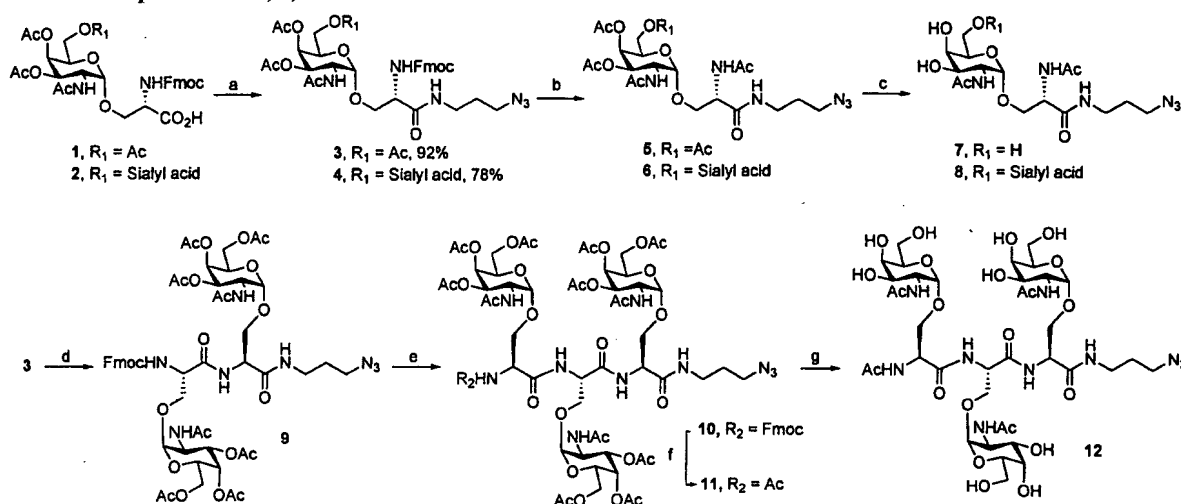
[a] Reagents and conditions: (a) HOBt, EDCI, H2N(CH2)3N3; (b) (i) 5% Et2NH/DMF, (ii) Ac2O; (c) 0.1 N NaOH in MeOH; (d) (i) 1% Et2NH/DMF, (ii) HOBt, EDCI, 1, 88%; (e) (i) 1% Et2NH/DMF, (ii) HOBt, EDCI, 1, 90%; (f) (i) 2% Et2NH/DMF, (ii) Ac2O, 96%; (g) 7 N NH3 in MeOH, 76%.

SCHEME 2. Synthesis of Modified Polypeptide (14)[a]
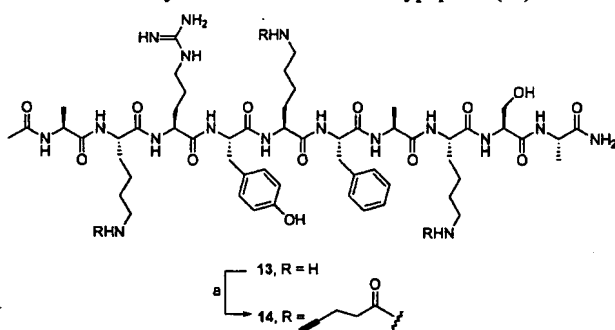
[a] Reagents and conditions: (a) *N*-succinimidyl-4-pentynoate, DMF, NaHCO3, H2O, 68%.

to the formation of glycopeptide—carrier protein conjugates, though application to the particular KLH carrier will be challenging for the reasons discussed above.

Under our modified conjugation strategy, an azido "handle" would be mounted on the carbohydrate, while the lysine residues of the carrier protein would be equipped with acetylene units (Figure 2). A 1,3-dipolar cycloaddition between the azide and alkyne would fashion the carbohydrate—carrier protein conjugate.

Results and Discussion

To evaluate in a preliminary way the feasibility of the proposed conjugation strategy, we prepared two monomeric

glycosylamino acids displaying the Tn (**7**) and STn (**8**) antigens, as well as a trimeric cluster composed of three Tn antigens (**12**) (Scheme 1). The synthesis of **7** commenced with the readily available carboxylic acid **1**.[12] Amide formation, as shown, allowed for the introduction of the azido group. Next, removal of the Fmoc group and capping of the amine with acetic anhydride, followed by global deprotection, produced coupling precursor **7**. The STn glycosylamino acid, **8**, was accessed from **2**[13] in a similar fashion.

The Tn cluster, **12**, was prepared from **3** through iterative Fmoc removal and coupling reactions with the monomeric unit, **1**. A series of standard manipulations provided **12**.

The surfaces of carrier proteins possess abundant lysine residues. To simulate the environment of the carrier protein, we designed a 10-mer polypeptide, **13**, possessing three lysine residues (Scheme 2). The acetylenic functional groups were introduced through exposure of **13** to *N*-succinimidyl-4-pentynoate in the presence of sodium bicarbonate.[14] Amide formation at the ε-amino groups of the lysine residues provided the desired polypeptide alkynes of **14** in good yield.

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SCHEME 3. Conjugation Adducts
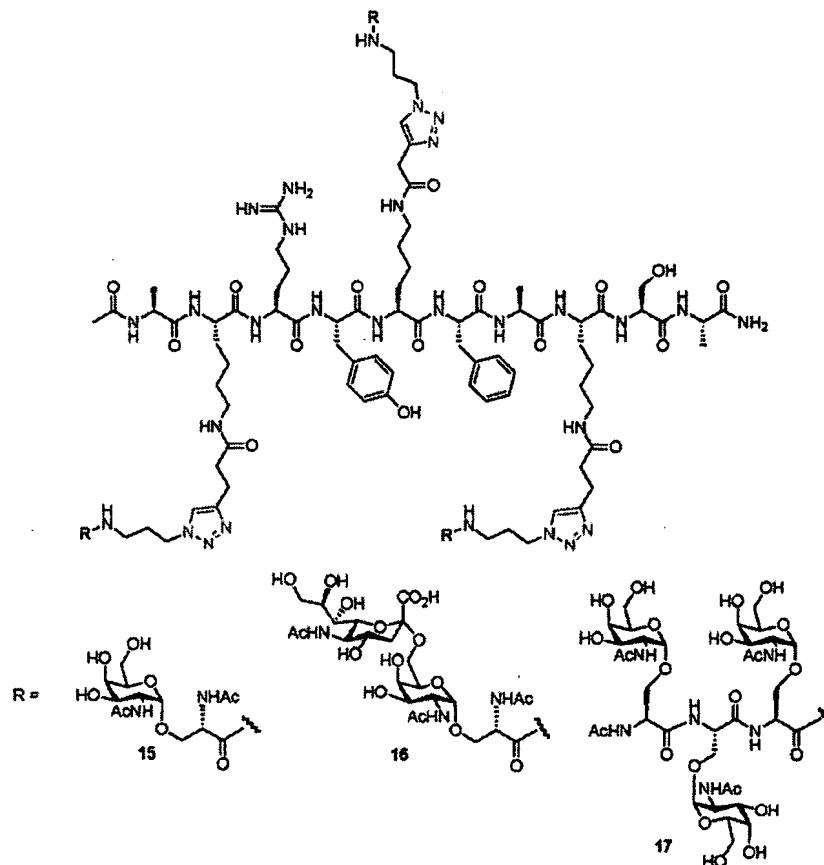
With the azido-carbohydrate and the alkynyl polypeptide components in hand, we investigated conditions to accomplish their merger. A variety of reaction conditions had already been reported for the generation of the active Cu[I] species.[15] For instance, the catalytic Cu(I) species could be introduced directly from the Cu(I) salt, most commonly from copper halide. However, in this case, prior exclusion of oxygen from the reaction is required in order to prevent the formation of undesired byproducts. Alternatively, Cu(I) can be generated through in situ reduction of Cu(II) with a variety of reducing agents, such as copper wire,[8] sodium ascorbate,[7a] and phosphine derivatives.[16] Several additives, such as tris-triazoles[17] and triethylamine hydrochloride,[18a] have been observed to significantly accelerate the cycloaddition event, presumably by stabilizing the catalytic Cu(I) species. An additional constraint imposed by our system was the need to operate in proper aqueous medium in order to ensure protein stability.

A number of ligation conditions were explored, with varying degrees of success. In our initial attempts, a combination of copper wire and CuSO₄ was utilized. Thus, to a solution of 7 and 14 in water and acetonitrile (1:1, v/v) was added polished

copper wire and a solution of CuSO₄ in water. The reaction was monitored by LC-MS, which indicated that the tris-triazole adduct (15, Scheme 3) was formed quantitatively after approximately 40 h. When sodium ascorbate was used as the reducing agent instead of copper wire, the reaction was found to be markedly less efficient.

Although we had identified conditions appropriate for the conjugation of the glycosylamino acid (7) with the polypeptide (14), we were not unmindful of the potential difficulties involved in translating this particular protocol to a protein setting. Thus, conjugation of the glycosylamino acid (or glycopeptide) to a carrier protein would require aqueous conditions and would be difficult to achieve in the presence of acetonitrile. Unfortunately, recourse to a purely aqueous solution from 50% acetonitrile resulted in a prohibitive decrease in reaction rate.

We next explored alternate means by which to gain access to Cu(I)—namely through oxidation of copper metal. In this regard, we found that, upon exposure to nanosized copper powder in water and *t*-BuOH (4:1 v/v), azide 7 and alkynyl peptide 14 underwent cycloaddition to provide the desired tris-triazole adduct (11) after 3 d (Figure 3a).[18b] We were surprised to find that addition of CuSO₄ to the reaction mixture actually had the effect of decreasing the reaction rate. We suspect that cycloaddition may actually occur on the surface of the nanosized copper,[19] which could be modified by the presence of CuSO₄.

To accelerate the conjugation rate and minimize the potential damage to the carrier protein caused by organic solvents, we

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a) Copper nano powder, 20% *t*-BuOH/H₂O, 1 day

b) Copper nano powder, PBS, 2 hours
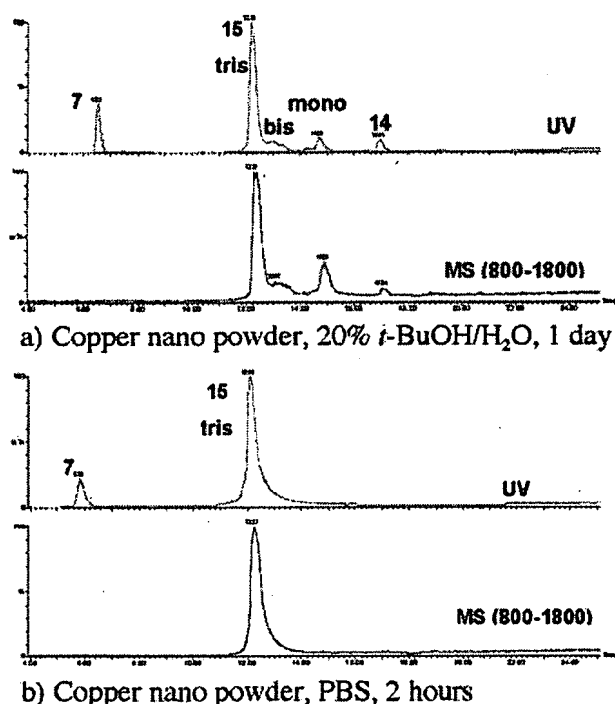
FIGURE 3. LC—MS traces of the [3 + 2] cycloaddition of 7 and 14.

performed the cycloaddition of 7 and 14 in water in the presence of Et₃N·HCl as an additive. Remarkably, it was found to greatly accelerate the cycloaddition reaction in aqueous *t*-BuOH.[18a] We were pleased to observe that each of three alkynyl moieties of 14 was transformed to a triazole over 3 h, and that the desired adduct was isolated in excellent yield. With the concern that Et₃N·HCl may have some detrimental effect on the carrier protein, the conjugation was carried out with nanosized Cu in the PBS (pH 7.2) without addition of Et₃N·HCl. We were pleased to find that the desired cycloaddition proceeded smoothly, at a rate comparable to that observed when the reaction was performed in the presence of Et₃N·HCl (Figure 3b). Although successful bioconjugations had been reported, additional ligands were employed in such conjugations.[8–10] To our knowledge, this is the first time that PBS alone has been reported to greatly enhance the reaction rate of the nanosized Cu-catalyzed Huisgen cyclization.

With these results in hand, we successfully applied the reaction conditions to the conjugation of the STn azide, 8, and the clustered Tn azide, 12. In each case, the appropriate tris-triazoles were formed cleanly[20] and in good yield in approximately 2 h.

In summary, we have reported herein the application of Sharpless-inspired protocols to the conjugation of fully synthetic
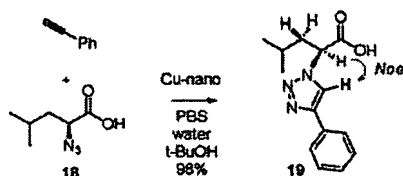
glycopeptide fragments to appropriately modified lysine residues. These mild, aqueous reaction conditions should be readily extendable to the conjugation of glycopeptide fragments to carrier proteins. This newly developed protocol represents a potentially powerful addition to the collection of conjugation techniques currently practiced, although admittedly application to the particular KLH case could remain problematic. While this application to the azide acetylene cycloaddition reaction is not yet a fully mature process, we expect that it will ease the path for the total synthesis and clinical evaluation of new vaccines directed to the enlistment of the formidable resources of the immune system in countering diseases. More broadly, this work is an exciting example of the potentiality in merging the teachings of chemistry and biology with a view to clinical application.

Experimental Section

AcHN-Tn(OAc)₃-CONH-(CH₂)₃-N₃ (5). To a solution of Tn-acid (1, 0.12 g, 0.18 mmol) in DMF—CH₂Cl₂ (1:1, 2.0 mL) at −20 °C was added HOBt (39.0 mg, 0.29 mmol) followed by EDCI (45.5 mg, 0.24 mmol). The reaction mixture was stirred at 0 °C for 30 min, and then H₂N—(CH₂)₃—N₃ (18.3 mg, 19.3 μL, 0.18 mmol) was added. After the mixture was stirred for 11 h, Et₂NH (100.0 μL) was added. The reaction mixture was stirred at room temperature for 10 h, and then Ac₂O (50.0 μL) was added. After being stirred overnight, the reaction mixture was diluted with EtOAc and washed with brine. The organic phase was dried with MgSO₄ and concentrated in vacuo. The residue was purified by silica gel column chromatography (2%, 5%, 10% gradient MeOH in CH₂Cl₂) to give the desired azide (5, 92.0 mg, 0.17 mmol, 90% over three steps) as a white foam: $[\alpha]^{22}_D = 70.6$ (*c* 1.0, CHCl₃); IR 3299, 3079, 2939, 2100, 1748, 1660, 1544, 1440, 1373, 1238, 1133, 1050, 916 and 732 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.94 (t, 1 H, *J* = 5.7 Hz), 6.66 (d, 1 H, *J* = 8.0 Hz), 6.24 (d, 1 H, *J* = 9.6 Hz), 5.33 (d, 1 H, *J* = 2.8 Hz), 5.06 (dd, 1 H, *J* = 3.2, 11.3 Hz), 4.83 (d, 1 H, *J* = 3.6 Hz), 4.66 (ddd, 1 H, *J* = 4.0, 4.5, 8.5 Hz), 4.54 (ddd, 1 H, *J* = 3.6, 9.6, 11.3 Hz), 4.10 (m, 2 H), 4.02 (dd, 1 H, *J* = 10.7, 6.5 Hz), 3.86 (dd, 1 H, *J* = 4.5, 10.1 Hz), 3.75 (dd, 1H, *J* = 4.5, 10.1 Hz), 3.35 (m, 4 H), 2.13 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 1.96 (s, 6 H), 1.77 (m, 2 H); ¹³C NMR (125 MHz, CDCl₃) δ 170.9, 170.8, 170.6, 170.5, 170.3, 169.5, 99.2, 69.2, 68.5, 67.2, 67.1, 61.8, 52.8, 49.3, 47.5, 37.4, 28.5, 23.2, 23.1, 20.73, 20.70; MS (EI) *m/z* 581.3 [M + Na]⁺; HRMS calcd for C₂₂H₃₅N₆O₁₁ [M + H]⁺ 559.2364, found 559.2358.

AcHN-Tn-CONH-(CH₂)₃-N₃ (7). To a solution of compound 5 (26.0 mg, 46.5 μmol) in MeOH (1.1 mL) was added aqueous NaOH (0.1 N, 1.1 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight before it was neutralized with Dowex and filtered. The residue was purified by HPLC (0−25% solvent B (CH₃CN with 0.04% TFA) in solvent A (H₂O with 0.05% TFA) over 20 min) to give the desired compound (7, 19.0 mg, 95%) as a white foam: ¹H NMR (500 MHz, D₂O) δ 4.87 (d, 1 H, *J* = 3.7 Hz), 4.50 (t, 1 H, *J* = 5.2 Hz), 4.13 (dd, 1 H, *J* = 3.7, 11.0 Hz), 3.96 (d, 1 H, *J* = 3.0 Hz), 3.90 (dd, 1 H, *J* = 4.6, 10.8 Hz), 3.85 (m, 2 H), 3.75 (dd, 1 H, *J* = 5.9, 10.8 Hz), 3.72 (m, 2 H), 3.34 (t, 2 H, *J* = 6.6 Hz), 3.28 (m, 2 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 1.76 (t, 1 H, *J* = 6.6 Hz), 1.73 (t, 1 H, *J* = 6.6 Hz); ¹³C NMR (125 MHz, D₂O + trace of acetone) δ 175.0, 174.8, 171.9, 98.4, 71.9, 68.9, 68.2, 67.8, 61.7, 54.6, 50.3, 49.1, 30.7, 28.2, 22.5, 22.2; MS (EI) *m/z* 455.3 [M + Na]⁺, 467.2 [M + Cl]⁻.

Compound 15. To a mixture of compound 7 (2.4 mg, 5.58 μmol) and compound 14 (1.8 mg, 1.24 μmol) were added PBS buffer (300.0 μL) and Cu(0) nanosize powder (1.0 mg) at room temperature. The reaction mixture was stirred for 2 h and then centrifuged for 5 min. The aqueous phase was removed, and the Cu(0) powder was washed with 500 μL of water and centrifuged. The aqueous

(20) The nanosized-Cu-catalyzed 1,3-dipolar cycloaddition of alkynes to azides in PBS buffer is a 1,4-regiospecific reaction. The regioselectivity was confirmed by NOE study. The synthesis of compound 18 followed a known literature precedent: Lundquist, J. T., IV; Pelletier, J. C. *Org. Lett.* 2001, 3, 781−783.

phases were combined and passed through a 0.2 μm filter. The light blue solution was purified by HPLC (5–35% solvent B in solvent A over 30 min, semi prep C18 column) to give the desired compound (15, 2.2 mg, 65%) as a white powder: selected ^{1}H NMR (500 MHz, D$_2$O) δ 7.76 (s, 2 H), 7.74 (s, 1 H), 7.31 (m, 2 H), 7.22 (m, 3 H), 7.05 (d, 2 H, *J* = 8.5 Hz), 6.79 (d, 1 H, *J* = 8.4 Hz), 6.77 (d, 1 H, *J* = 8.4 Hz), 4.87 (d, 3 H, *J* = 3.7 Hz), 2.09 (s, 3 H), 2.08 (s, 9 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 2.00 (s, 9 H); MS (EI) *m/z* 1375.2 [M + 2H]$^{2+}$.

Supporting Information Available: Experimental procedures, NMR spectra, and characterization for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Combining ATRP and "Click" Chemistry: a Promising Platform toward Functional Biocompatible Polymers and Polymer Bioconjugates

Jean-François Lutz,*,† Hans G. Börner,‡ and Katja Weichenhan†

Research Group Nanotechnology for Life Science, Fraunhofer Institute for Applied Polymer Research, Geiselbergstrasse 69, Golm 14476, Germany, and Colloid Department, Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany

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ABSTRACT: The bromine chain-ends of well-defined poly(oligo(ethylene glycol) acrylate) (POEGA) (M_n = 6850 g·mol^{-1}, M_w/M_n = 1.21) prepared using ATRP were successfully transformed into various functional end groups (ω-hydroxy, ω-amino, and ω-Fmoc-amino acid) via a two step pathway: (1) substitution of the bromine terminal atom by an azide function, (2) 1,3-dipolar cycloaddition of the terminal azide and functional alkynes (propargyl alcohol, propargylamine, and N-α-(9-fluorenylmethyloxycarbonyl)-L-propargylglycine). The efficient "click" cycloaddition was confirmed in all cases by ^{1}H NMR or SEC−UV analysis. Moreover, this two-step synthetic strategy was also studied for preparing polymer-*b*-oligopeptide bioconjugates. Well-defined POEGA-*b*-GGRGDG was obtained in high yields via the "click" ligation of the azido functional POEGA and the alkyne functional oligopeptide GGRGDG.

Introduction

Synthetic biocompatible polymers are crucial materials in modern biomedical applications.[1,2] For instance, water-soluble polymers, stimuli-responsive polymers, or polymer bioconjugates are extensively applied in various biotechnological areas such as pro-drug systems, protein delivery, gene therapy, bioassays, or bioseparation.[1−5] In this context, the development of novel synthetic methods at the interface of standard polymer chemistry and biochemistry is particularly important.

Recently, Sharpless et al. popularized in organic synthesis the 1,3-dipolar cycloaddition of azides and terminal alkynes, catalyzed by copper(I).[6] Such reactions were proven to be very versatile, since they can be performed in high yield, in multiple solvents (including water) and in the presence of numerous other functional groups. Hence, these cycloadditions were evidenced to be efficient tools for the ligation of synthetic organic molecules and biological structures such as peptides, proteins, viruses or cells.[7−14] Due to their efficiency and simplicity, azide/alkyne cycloadditions were ranked by Sharpless in the category of "click" reactions.[15]

During the last 2 years, "click" cycloadditions became also very popular in polymer chemistry, as a tool for functionalizing synthetic macromolecules.[16−41] The transfer of "click" chemistry in polymer synthesis first started with the very influential works of Fréchet and Hawker. Their early publications in the field illustrated that the 1,3-dipolar cycloaddition of azides and alkynes is a promising reaction for preparing either dendrimers[20,24,27,35] or functional linear polymers.[18,28] Shortly after, we and others reported the combination of "click" chemistry and atom transfer radical polymerization (ATRP).[26,30,34] Such a step was important since ATRP is probably one of the most employed polymerization method in modern material science.[42−44] Indeed, ATRP is a facile technique, which allows the preparation

of well-defined polymers with narrow molecular weight distribution, predictable chain length, controlled microstructure, defined chain-ends and controlled architecture.[44−50] Nevertheless, the range of possibilities of ATRP can be further broadened by "click" chemistry. For example, the ω-bromine chain-ends of polymers prepared by ATRP can be transformed into azides by nucleophilic substitution and subsequently reacted with functional alkynes.[51,52] Such approach was used for preparing either well-defined telechelic polymers or block copolymers.[21,22,26,30,34] Additionally, functional initiators or monomers (i.e., azide or alkyne functional molecules) can be used in ATRP for preparing well-defined "clickable" polymers.[29,30,33,34,38]

Such combination of "click" chemistry and ATRP could be further developed as an interesting platform for building tailor-made biomaterials. However, so far, only a few steps were made in this direction,[21,38] even though, as mentioned above, "click" chemistry appears to be an interesting link between the synthetic and biological worlds.

Poly(ethylene glycol) (PEG) is undoubtedly the most studied and applied synthetic polymer in the biomedical field.[1] Indeed, PEG is an uncharged, water-soluble, nontoxic, nonimmunogenic polymer and therefore an ideal material for bioapplications. Classic examples of application include peptide/protein PEGylation, stealth drug carriers, and protein repellent surfaces.[5,53,54] In general, PEGylation chemistry relies on short end-functional linear PEG macromolecules. However, it was recently demonstrated than nonlinear PEG analogues, possessing a vinyl backbone and multiple PEG side chains, can also be used for building advanced biomaterials.[55−65] Such structures can be obtained via either radical (i.e., free radical polymerization or controlled radical polymerization techniques such as ATRP) or anionic polymerization of oligo(ethylene glycol) macromonomers.[66−70] Recent studies indicated that these brushlike PEG macromolecules are as biocompatible (e.g., nontoxic, nonimmunogenic) as their linear counterpart.[55,57,58,60−65] Moreover, nonlinear PEG analogues can exhibit new properties, which are typically not attainable with linear PEG. For example, we recently reported that the copolymerization of two oligo-

* Corresponding author. E-mail: lutz@iap.fhg.de.
† Research Group Nanotechnology for Life Science, Fraunhofer Institute for Applied Polymer Research.
‡ Colloid Department, Max Planck Institute of Colloids and Interfaces.

Scheme 1. Preparation of ω-Azide Functional Poly(oligo(ethylene glycol) acrylate) (POEGA) and Molecular Structures of the Various Alkynes Studied Herein for "Click" Functionalization of POEGA
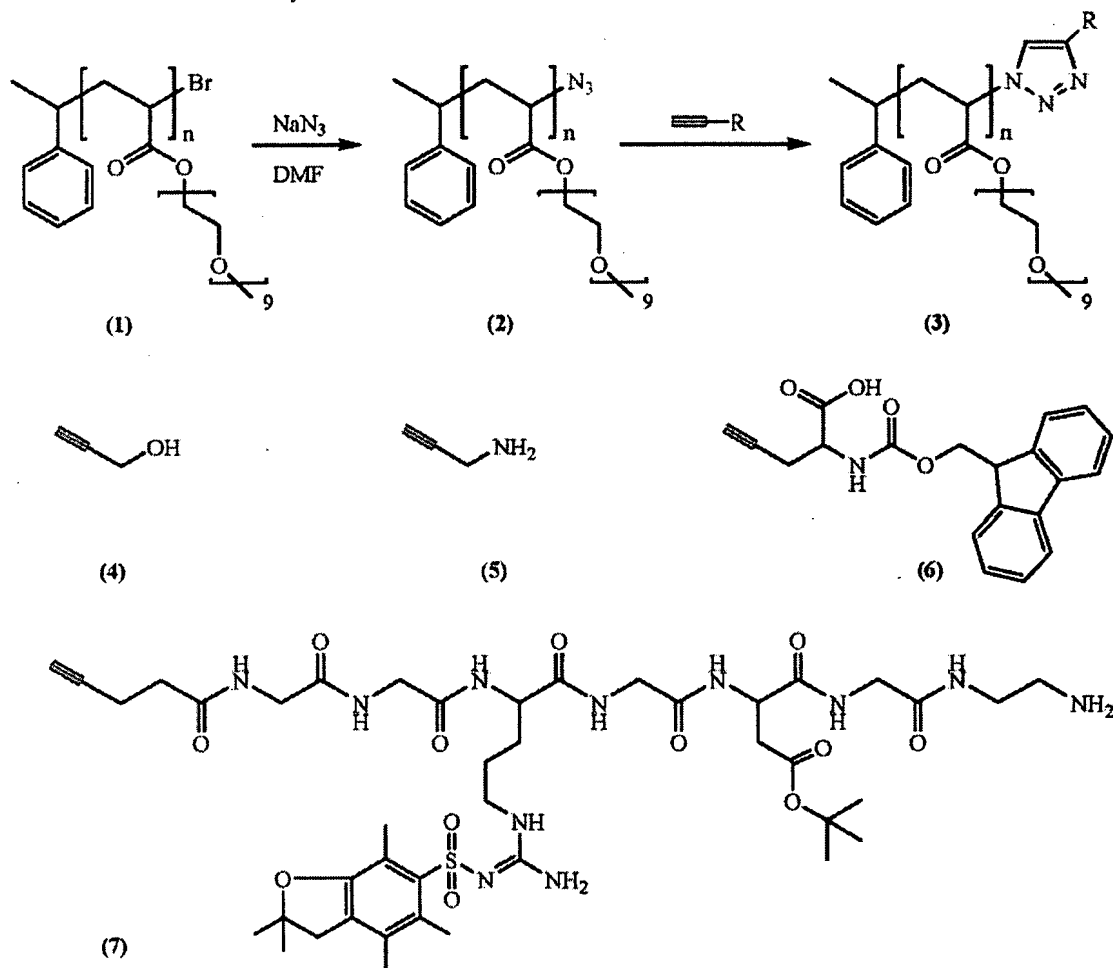
(ethylene glycol) macromonomers of different chain-lengths lead to the formation of thermosensitive copolymers with a precisely tunable lower critical solution temperature (LCST).[71,72]

In the present article, we studied the 1,3-dipolar cycloaddition of azides and terminal alkynes as a general methods for functionalizing nonlinear PEG macromolecules prepared using ATRP (Scheme 1). The ω-chain-end of well-defined azide-terminal poly(oligo(ethylene glycol) acrylate) (POEGA) was studied as a reactive site for "click" coupling. This approach was preferred to the use of α-functional initiators (e.g., alkyne or azide functional initiators) since the ω-functionality is the only one available when polymers are grown from a surface (e.g., from inorganic nanoparticles or from flat substrates).[50] Our chemistry is therefore adapted for the post-functionalization of biocompatible surfaces. As a proof of concept, the "click" ligation of ω-azide functional POEGA to either low molecular weight functional alkynes or sequence-defined oligopeptides is described herein.

Experimental Part

Chemicals. Propargyl alcohol (Aldrich, 99%), propargylamine (Aldrich, 99%), oligo(ethylene glycol) methyl ether acrylate (Aldrich, M_n = 454 g·mol⁻¹), 2,2'-bipyridyl (Bipy) (Fluka, 98%), N,N,N',N',N'' pentamethyldiethylenetriamine (PMDETA) (Aldrich, 99%), 4,4'-Di-(5-nonyl)-2,2'-bipyridine (dNbipy) (Aldrich, 99%), (1-bromoethyl) benzene (Acros, 97%), and sodium azide (Aldrich, 99%) were used as received. Copper(I) bromide (Acros, 95%) was

washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol, and dried. N-α-(9-Fluorenylmethyloxycarbonyl)-L-propargylglycine (Fmoc-L-pra-OH), N-α-(9-fluorenylmethyloxycarbonyl)-N'-2,2,4,6,7-pentameth-yldihydrobenzofuran-5-sulfonyl-L-arginine (Fmoc-L-Arg(Pbf)-OH), N-α-(9-fluorenyl- methyloxycarbonyl)glycine (Fmoc-Gly-OH), N-α-(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid β-*tert*-butyl ester (Fmoc-L-Asp(*t*Bu)-OH), and the polystyrene-(2-chlorotrityl chlo-ride) resin (loading: 0.25 mmol/g) were received from Iris Biotech GmbH (peptide grade).

Preparation of Bromine End-Functional Poly(oligo(ethylene glycol) acrylate) 1 via Atom Transfer Radical Polymerization. Copper bromide (CuBr) (172 mg, 1.20 mmol) and 2,2'-bipyridyl (Bipy) (375 mg, 2.40 mmol) were added into a dry flask. The flask was sealed with a septum and subsequently purged with dry argon for several minutes. Then, 20 mL of thoroughly degassed oligo-(ethylene glycol) methyl ether acrylate (Aldrich, M_n = 454 g·mol⁻¹) was added with a degassed syringe. The mixture turned dark brown, indicating complexation of Cu^{I}Br and Bipy. Last, (1-bromoethyl) benzene (222 mg, 1.20 mmol) was added with a precision syringe. The mixture was heated at 90 °C in an oil bath. After 3 h, the experiment was stopped by opening the flask and exposing the catalyst to air. The final mixture was diluted with deionized water and subsequently purified by dialysis against pure water (Roth, ZelluTrans membrane, molecular weight cutoff: 4000−6000). Last, water was removed by rotary evaporation. The purified polymer appeared as a clear yellowish oil.

Preparation of Azide End-Functional Poly(oligo(ethylene glycol) acrylate) 2. The procedure for transforming bromide

ω-functional POEGA into azide ω-functional POEGA was adapted from the literature.[52] Typically, the bromine end-functional poly-(oligo(ethylene glycol) acrylate) **1** (6850 g·mol⁻¹) (5.5 g, 0.80 mmol), sodium azide (57 mg, 0.88 mmol) and dimethylformamide (6 mL) were added in a dry flask. The clear homogeneous solution was stirred at room temperature for 3 h. The final mixture was diluted with deionized water and subsequently purified by dialysis against pure water (Roth, ZelluTrans membrane, molecular weight cutoff: 4000−6000). Last, water was removed by rotary evaporation.

General Procedure for the "Click" Coupling of Azide End-Functional Poly(oligo(ethylene glycol) acrylate) 2 and a Low Molecular Weight Functional Alkyne (either 4, 5, or 6). In a flask, polymer **2** (200 mg, 0.03 mmol), copper bromide (21 mg, 0.14 mmol) and 2 mL of tetrahydrofuran (THF) were added. The flask was capped with a septum and the solution was purged with dry argon for 5 min. Then, 4,4′-di(5-nonyl)-2,2′-bipyridine (dNbipy) (119 mg, 0.29 mmol) dissolved in 3 mL of degassed THF was added with a degassed syringe through the septum and the mixture turned to a homogeneous brown/dark red solution. Last, the functional alkyne (0.29 mmol) was added via a microliter syringe (only alkynes **4** and **5** are liquid—the solid alkyne **6** was added at the beginning of the reaction, together with CuBr). In all cases, the reaction mixture was stirred for 24 h at room temperature. After reaction, the final mixture was diluted in tetrahydrofuran (THF) and passed through a short silica column in order to remove copper catalyst. Then, the THF solution was further diluted with an excess deionized water and subsequently purified by dialysis against pure water (Roth, ZelluTrans membrane, molecular weight cutoff: 4000−6000). Last, water was removed by rotary evaporation.

Synthesis of the Alkyne Functional Oligopeptide GGRGDG 7. The solid-phase supported peptide synthesis of **7** was performed on an Applied Biosystems ABI 433a peptide synthesizer using *N*-methyl-2-pyrrolidone (NMP) as solvent. The coupling of the amino acids was facilitated by HBTU/DIPEA and Fmoc protocols were followed as described previously.[73,74] A polystyrene-(2-chlorotrityl chloride) resin was preloaded with 1,2-ethanediamine via standard procedures[75] and subsequently used for attaching the Fmoc-amino acid derivatives to give the peptide GGRGDG. After removal of the final Fmoc group, the N-terminal amine group was amidated with 20 equiv excess of 4-pentynoic acid using standard DCC coupling protocols. **7** was liberated from the support by twice treatment with a cleavage mixture (2 vol % of trifluoroacetic acid (TFA) in dichloromethane) for 2 min, followed by washing cycles. After diethyl ether precipitation, centrifugation, and lyophilization from 50 vol % water/acetonitrile **7** was isolated in about 78% yield. ESI−MS: $C_{42}H_{65}N_{11}O_{12}S$, $M_{th} = 947.45$ Da; 948 *m/z* ([M + H]⁺); 475 *m/z* ([M + 2H]²⁺); 447 *m/z* ([M-*t*Bu + 2H]²⁺).

General Procedure for the "Click" Coupling of Azide End-Functional Poly(oligo(ethylene glycol) acrylate) 2 and the Alkyne Functional Oligopeptide GGRGDG 7. Polymer **2** (100 mg, 0.015 mmol), the oligopeptide **7** (55 mg, 0.06 mmol), and copper bromide (10 mg, 0.07 mmol) were added together into a dry flask. The glassware was capped with a septum and purged with dry argon for several minutes. Then, Bipy (23 mg, 0.14 mmol) dissolved in 2 mL of degassed *N*-methyl pyrrolidone (NMP) was added via a degassed syringe. The mixture turned dark brown, indicating complexation of Cu(I)Br and Bipy. The reaction mixture was stirred for 24 h at room temperature. The final mixture was diluted in deionized water and subsequently purified by dialysis against pure water (Roth, ZelluTrans membrane, molecular weight cutoff: 4000−6000). Last, water was removed by rotary evaporation.

Measurements and Analysis. Size Exclusion Chromatography, SEC. (1) Molecular weights and molecular weight distributions were determined by SEC performed in THF as eluent and using three 5 μ-MZ-SDV columns with pore sizes of 10³, 10⁵, and 10⁶ Å (flow rate 1 mL·min⁻¹). The detection was performed with a RI−(Shodex RI-71). For calibration, linear polystyrene standards (PSS, Germany) were used. (2) Sanger tests SEC monitoring were performed at 20 °C using a Waters 515 HPLC isocratic pump

Table 1. Molecular Description of the Well-Defined POEGA Prepared by ATRP[a]

monomer	*t* (h)	conv	M_n GPC	M_w/M_n	M_n NMR	$M_{n\,th}$[b]
1 OEGA	3	0.33	6850	1.21	7200	6000

[a] Experimental conditions: bulk, 60 °C, [OEGA]₀/[PEBr]₀/[CuBr]₀/[Bipy]₀ = 40/1/1/2. [b] $M_{n\,th}$ = conv. (454 [OEGA]₀/[PEBr]₀).

equipped with a Waters 2414 Refractive Index detector, a Waters 2487 UV detector (set at a wavelength of 380 nm) and a set of Styragel columns (HR5, HR 45, HR 3, 500−100 000 Da) from Waters. Eluent: THF (HPLC, from Roth). Flow rate: 1 mL·min⁻¹. The degree of functionalization of ω-amino functional polymers was roughly estimated by comparing the intensities of the polymer signal and the signal of the unreacted Sanger reagent.

¹H NMR. ¹H NMR spectra were recorded at room temperature in acetone-d_6 or dimethyl sulfoxide-d_6 (DMSO-d_6) on a Bruker DPX-400 operating at 400.1 MHz. Monomer conversions were calculated from ¹H NMR spectra by comparing the integrations of the vinyl protons of the remaining monomer (5.90, 6.18, and 6.35 ppm) to the overall integration of the region 3.90−4.70 ppm where 2 protons of the remaining monomer and 2 protons of the formed polymer resonate.

Results and Discussion

A combination of ATRP and "click" chemistry was studied as a new strategy for preparing functional PEG analogues and PEGylated oligopeptides. Poly(oligo(ethylene glycol) acrylate) (POEGA) was chosen as model polymer in this study. The acrylate macromonomer (oligo(ethylene glycol) acrylate) (OEGA) was preferred to its methacrylate counterpart since the nucleophilic substitution of halogen end groups into azides was reported to be generally faster for poly(acrylates) than for poly(methacrylates).[45,52]

A well-defined POEGA sample **1** was prepared by bulk ATRP in the presence of the catalyst system Cu(I)Br/Bipy (Table 1). For this reaction, the initiator (1-bromoethyl) benzene (PEBr) was selected, since the phenyl protons of this moiety can be clearly detected and quantified in the ¹H NMR spectrum of POEGA.[76] As described in the literature, the polymerization was stopped at rather low monomer conversion in order to obtain a polymer with a high degree of terminal halogen functionality.[77,78] Previous reports indicated that at this stage of the polymerization (i.e., ~30% monomer conversion), the bromine end-functionality of poly(acrylates) prepared by ATRP is above 90%.[76,78] However, after polymerization, the reaction mixture still contained a high proportion of unreacted OEGA. This excess of macromonomer was selectively separated form the polymer via dialysis in pure water as shown in Figure 1. Indeed, the molecular weight distribution of POEGA was slightly narrowed in this process since a fraction of the low molecular weight chains is lost during dialysis. Nevertheless, after purification, a well-defined POEGA was obtained (M_n = 6850 g·mol⁻¹, M_w/M_n = 1.21). The number-average molecular weight determined by SEC in THF for this sample was also confirmed by ¹H NMR (i.e., by comparing the integration of the protons of the initiator and the protons of the backbone). Nevertheless, number-average molecular weight and molecular weight distribution of the polymer were not affected by further dialysis steps using the same type of membranes (Figure 1).

To perform a 1,3-dipolar "click" cycloaddition azide/alkyne at the ω-chain-end, the bromo functional POEGA **1** was transformed in an azide ω-functional polymer via nucleophilic substitution with sodium azide, as described previously in the literature.[52] The resulting POEGA **2** was subsequently involved in "click" reactions with various low molecular weight functional alkynes (propargyl alcohol, the amino acid *N*-α-(9-
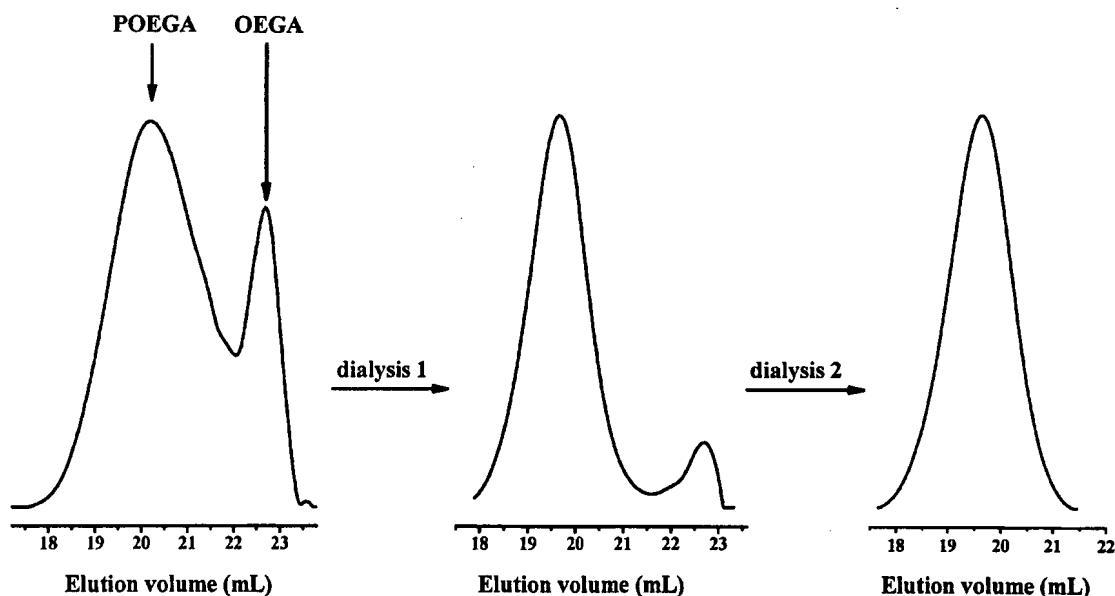
Figure 1. SEC chromatograms recorded in THF for a well-defined poly(oligo(ethylene glycol) acrylate) (POEGA) prepared by ATRP. Left: raw POEGA **1** at the end of the polymerization. Middle: purified POEGA **1** after dialysis in water. Right: POEGA **2** after reaction with NaN₃ and purification by dialysis in water.

fluorenylmethyloxycarbonyl)-L-propargylglycine (Fmoc-L-pra-OH) and propargylamine) in order to prepare functional PEG analogues. Typically, in the absence of an appropriate catalyst, the reaction between azides and terminal alkynes is quite slow since these alkynes are poor 1,3-dipole acceptors. However, in the presence of copper(I), which can bind to terminal alkynes, the cycloaddition reactions are dramatically accelerated, regio-selective and highly efficient (yields are often above 95%). In the present work, copper(I) bromide and either the bidentate ligand 4,4'-di(5-nonyl)-2,2'-bipyridine (dNbipy) or the tridentate ligand PMDETA were used as catalyst for the azide/alkyne cycloaddition. Both combinations were selected since they lead to homogeneous reaction mixtures in THF.[22,26]

Figure 2 compares the ¹H NMR spectra measured for the azide ω-functional POEGA **2** before and after reaction with propargyl alcohol. The chain-ends of this polymer can be detected and quantified using NMR. From 7.05 to 7.40 ppm, a broad signal due to the aromatic protons of the initiator moiety (Hₐ) can be observed. After cycloaddition with propargyl alcohol, two new chain-ends signal appeared in the NMR spectrum. The signal at 4.50−4.80 ppm corresponds to the two methylene protons neighboring the terminal alcohol function (Hₑ) whereas the signal at 7.90−8.10 ppm was assigned to the proton of the triazole ring.[26] The integration of the signals due to α- and ω- chain-ends allows calculation of the fraction of chains initiated by (1-bromoethyl) benzene and terminated by an hydroxy methylene triazole. Degree of functionalization higher as 90% could be obtained (Figure 2B). It is important to remind that this number cannot reach 100% since atom transfer radical polymerizations are, by essence, subject to termination reactions.[77,78] The latter can be minimized but never completely suppressed. Nevertheless, these results indicate that the two chain-end modification steps (i.e., transformation into azide and "click" cycloaddition) are most likely quantitative as previously evidenced for polystyrene and poly(acrylate) models.[26,37]

The functionalization of synthetic polymers with Fmoc-protected amino acid moieties is an important issue since it would open the possibility of using short macromonomers in solid-phase peptide synthesis. Figure 2C shows the ¹H NMR spectra measured for a purified POEGA after reaction with

Fmoc-L-pra-OH. The apparition of several broad signals between 7 and 8 ppm confirmed the efficient formation of an ω-amino acid POEGA. This particular region contains the aromatic protons of the initiator moiety, the proton of the triazole and the protons of the fluorene ring in Fmoc. In particular, the region 7.6−8.0 ppm contains the four most deshielded protons of the Fmoc moiety. Nevertheless, the proton of the triazole ring could not be separately observed in this spectrum. However, due to the Fmoc-amino acid neighboring group, this proton is theoretically expected to be more shielded than its counterpart in Figure 2B.

The functionalization of POEGA **2** with propargylamine was a less challenging target since terminal azides can also be directly reduced to primary amine via iminophosphorane intermediates.[79] Nevertheless, this reaction was investigated in order to illustrate further the versatility of "click" cycloadditions in polymer synthesis. However, the formed ω-primary amino functional POEGA could not be studied by ¹H NMR since the characteristic protons of the ω-chain-ends overlap with other signals. Thus, the existence of ω-amino end groups was confirmed by a standard colorimetric test. 1-Fluoro-2,4-dini-trobenzene (also known as the Sanger reagent) reacts selectively with primary amines via nucleophilic aromatic substitution, resulting in an intense yellow chromophore (Figure 3). The latter was effectively observed when ω-amino POEGA was put in the presence of the Sanger reagent in pure THF (control THF solutions are colorless). However, such visual test should not be overinterpreted since the yellowing could also result from the presence of low molecular weight impurities (e.g., traces of unreacted propargylamine). To rule out this possibility, the POEGA sample reacted with the Sanger reagent was investi-gated by SEC−UV (Figure 3). This measurement indicated the presence of primary amines functions at the ω-chain-ends of POEGA (the differences in elution volumes between Figures 1 and 3 are due to the use of two different SEC setups). Moreover, the signal intensity confirmed a high degree of chain-end functionalization.

The bioconjugation of nontoxic, nonimmunogenic polymers such as PEG with peptides, enzymes or proteins is an important aspect of modern biotechnology.[5] However, Haddleton and co-
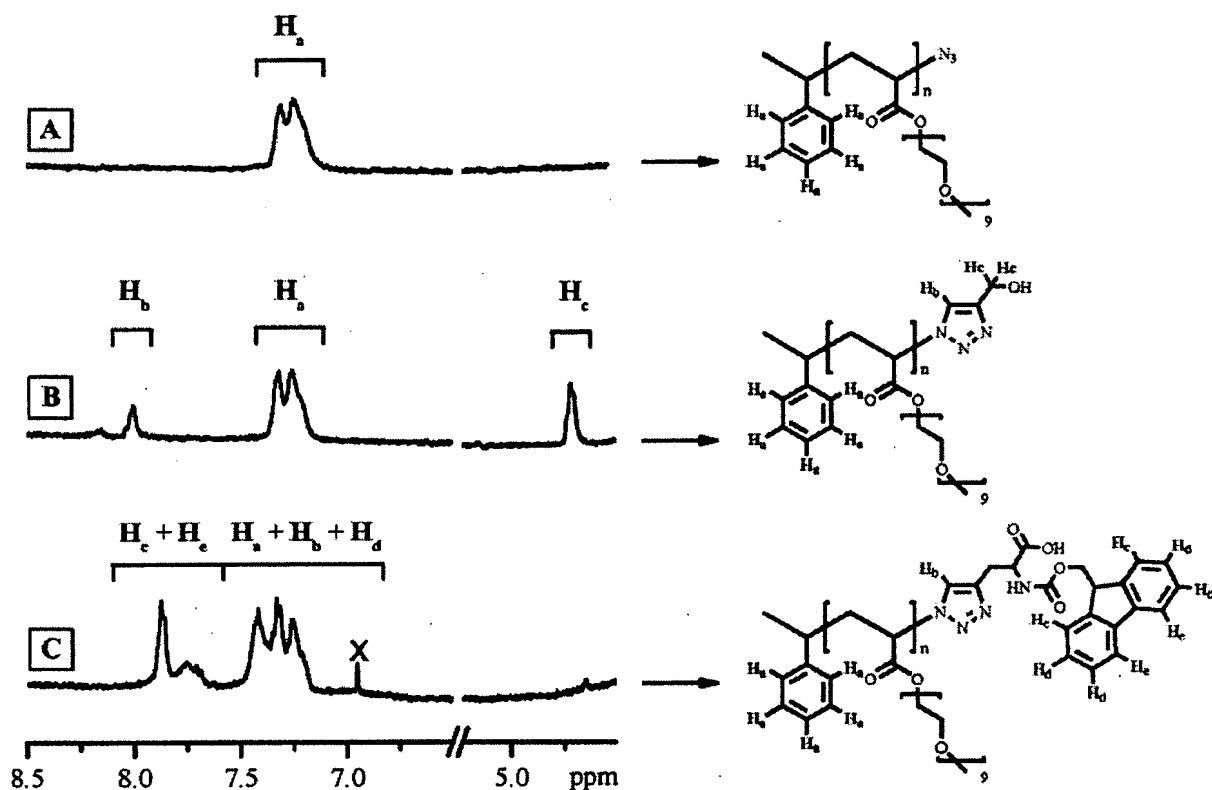
Figure 2. ¹H NMR spectrum (zooms of the regions 8.5–6.5 ppm and 5.2–4.5 ppm) of purified poly(oligo(ethylene glycol) acrylate) (POEGA): (A) azide ω-functional before "click" cycloaddition, (B) after "click" cycloaddition with propargyl alcohol, (C) after "click" cycloaddition with *N*-α-(9-fluorenylmethyloxycarbonyl)-L-propargylglycine. All spectra were recorded at room temperature in acetone-*d₆*.
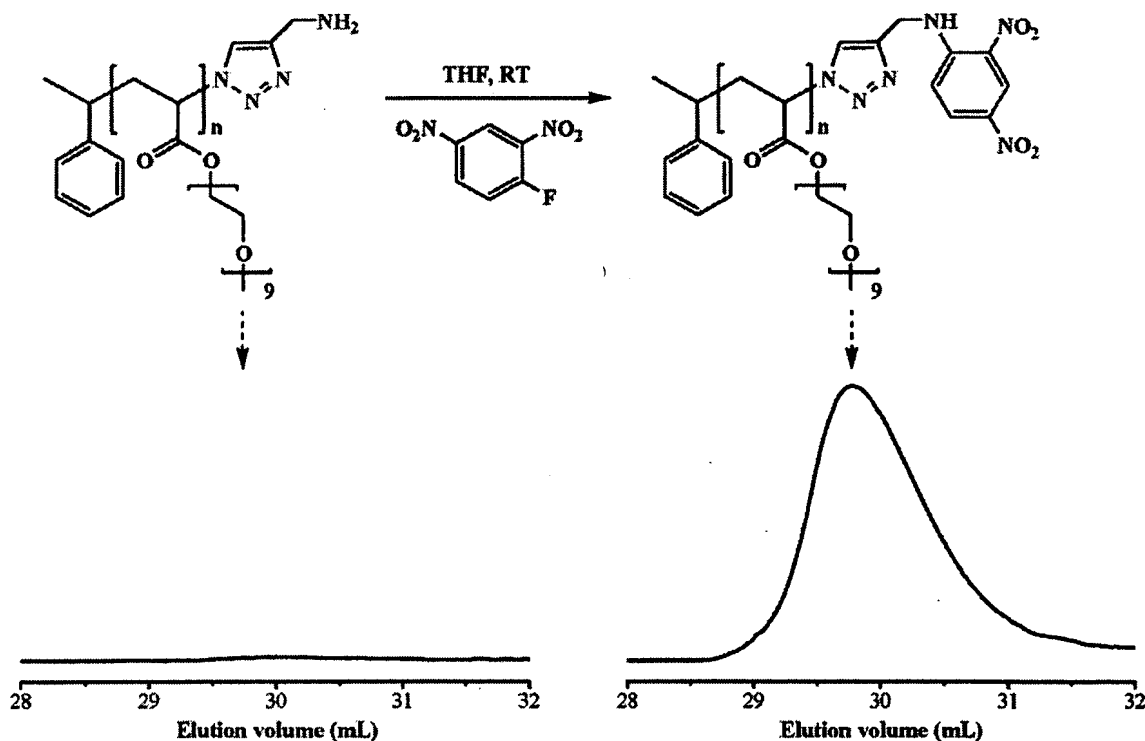
Figure 3. Schematic representation of a primary amine test using 1-fluoro-2,4-dinitrobenzene (Sanger reagent) and SEC chromatograms measured for ω-amino functional poly(oligo(ethylene glycol) acrylate) (POEGA) before and after reaction with the Sanger reagent. SEC chromatograms were recorded in THF with the help of UV detector set at a wavelength of 380 nm.

workers already demonstrated that macromolecules constructed with PEG macromonomers are promising alternative to standard linear PEG for the bioconjugation of proteins.[29,59] It was therefore important to expand our synthetic strategy for the preparation of well-defined bioconjugates of POEGA and oligopeptides.
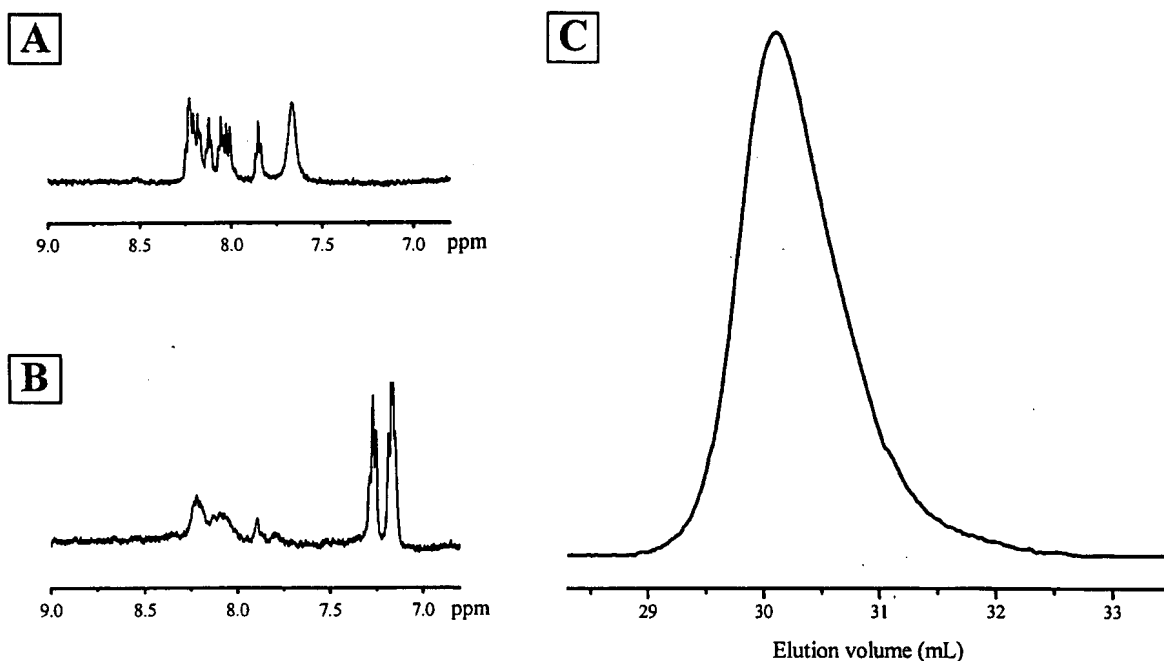
Figure 4. ^{1}H NMR spectrum (zooms of the region 9—6.8 ppm) recorded at room temperature in DMSO-d_6 for (A) the oligopeptide GGRGDG **7** and (B) poly(oligo(ethylene glycol) acrylate) (POEGA) after "click" cycloaddition with the oligopeptide **7**. (C) SEC chromatogram measured for ω-GGRGDG functional poly(oligo(ethylene glycol) acrylate) (POEGA) after reaction with the 1-fluoro-2,4-dinitrobenzene. The chromatogram was recorded in THF with the help of UV detector set at a wavelength of 380 nm.

Many cell receptors of the integrin family recognize the arginine-glycine-aspartic acid (Arg-Gly-Asp, RGD) segment within their ligands.[80] These ligands include fibronectin, fibrinogen, and other large glycoproteins.[81] Recently, it was demonstrated that the RGD-labels can be applied in a versatile manner to enhance the bioacceptability and the biocompatibility of surfaces[82,83] and to induce targeting of drugs or colloidal drug carriers,[84] as well as to control cell adhesion on synthetic materials.[85] Hence, the RGD motif was selected in the present study as a model oligopeptide for the "click" ligation with POEGA.

The oligopeptide GGRGDG **7** was prepared by solid-phase supported synthesis using standard Fmoc protection chemistry. While still on the solid support, the terminal amino-functionality of the polypeptide was transformed in an alkyne moiety via a coupling reaction with 4-pentynoic acid in the presence of N,N'-dicyclohexylcarbodiimide. The protecting groups of the arginine and aspartic acid amino acids were kept in the molecular structure after synthesis to allow sufficient THF solubility and therefore a reliable SEC analysis of the polymer bioconjugate. Additionally, the oligopeptide was functionalized with a primary amine end group (obtained when the peptide was cleaved from the solid support) to allow further coupling with the Sanger reagent and SEC—UV analysis.

The "click" reaction of POEGA **2** and the oligopeptide **7** was investigated in N-methyl pyrrolidone in the presence of the catalyst system Cu(I)Br/Bipy. As expected, the coupling between the azido functional POEGA and the alkyne functional GGRGDG occurred, as evidenced by ^{1}H NMR and SEC—UV. Parts A and B of Figure 4 compare the region 9—6.8 ppm of the ^{1}H NMR spectra measured respectively for the pristine oligopeptide **7** and the formed bioconjugate POEGA-b-GGRGDG after purification. The broad signals in Figure 4A are solely due to the secondary amine backbone protons of the oligopeptide. These protons still appear in Figure 4B, thus confirming the presence

of the oligopeptide moiety in the bioconjugate POEGA-b-GGRGDG.

The integration of the secondary amine protons and the initiator protons in Figure 4B indicated that approximately 75% of the POEGA chains were functionalized by the oligopeptide. This degree of functionalization is lower than the one observed for low molecular weight alkynes and could be explained by steric hindrance of the functional macromolecules. However, it is noteworthy that secondary amine protons are usually leading to broader and weaker ^{1}H NMR signals in comparison to C—H protons. Hence, the number calculated above might also be slightly underestimated.

Nevertheless, the formation of a well-defined bioconjugate POEGA-b-GGRGDG was further confirmed by the Sanger test and subsequent SEC—UV—RI analysis. Figure 4C shows the chromatogram measured for POEGA-b-GGRGDG after treatment with 1-fluoro-2,4-dinitrobenzene. A monomodal well-defined polymer could be observed with both UV and RI detectors. In comparison, the pristine bioconjugate POEGA-b-GGRGDG (i.e., not reacted with the Sanger reagent) gave no UV signal at the studied wavelength. Moreover, SEC confirmed the complete removal of the excess of the oligopeptide **7** during the purification process.

Conclusion

The 1,3-dipolar cycloaddition of azides and terminal alkynes was investigated as a general tool for functionalizing the ω-chain-ends of well-defined poly(oligo(ethylene glycol) acrylate) prepared using ATRP. This synthetic approach permitted to functionalize "on demand" the polymer with either low molecular weight motifs (e.g., ω-hydroxy, ω-amino or ω-amino acid terminal functions) or sequence-defined oligopeptides. These new results confirm our previous study on well-defined polystyrene models and highlight that the combination of ATRP and click chemistry is a facile and versatile method for preparing

functional macromolecules.[26] Moreover, the synthetic strategy reported in this article can be potentially considered as an "universal" route toward the design of novel biomaterials such as tailor-made polymer bioconjugates and functional biosurfaces.

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Click Chemistry – What's in a Name? Triazole Synthesis and Beyond

María Victoria Gil,*[a] María José Arévalo,[a] Óscar López[b]

[a] Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Extremadura, 06071 Badajoz, Spain
Fax +34(924)271149; E-mail: vgil@unex.es

[b] Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, 41012 Seville, Spain

Abstract: The environmentally amiable route to carbon–heteroatom bond formation, described by Sharpless as 'click chemistry', has become known as a fast, efficient, and reliable approach to the synthesis of novel compounds with desired functionalities. Readily available starting materials must be used in this methodology and they should be essentially inert to most biological and organic conditions, including water and molecular oxygen. In this review, we cover reactions included in this label such as cycloadditions, nucleophilic ring-opening reactions of strained cycles, and amide synthesis, as well as their applications in organic synthesis, molecular biology, macromolecular chemistry and materials science.

1   Introduction

Modern research in organic chemistry and pharmacological areas require the preparation of structurally complex molecules through a rational design; furthermore, if these molecules are to have any practical applications, the synthetic route should be both synthetically and economically viable. Although combinatorial chemistry emerged as a powerful tool to provide extensive libraries of compounds, applications in the pharmaceutical field usually depend strongly on the success of individual reactions.[1] In that sense, a change in the way of considering synthetic challenges was needed in order to provide reliable reactions; this change took place in 2001 when the term 'click chemistry' was coined by Sharpless[2] in an attempt at obtaining chemical diversity suitable for biological screening, by means of rapid and efficient protocols.

Click chemistry refers to a series of reactions that obey certain criteria, such as being modular, stereospecific, high-yielding and involving simple experimental procedures.[2,3] They should also make use of readily available starting materials, environmentally friendly conditions (water as solvent, or solvent-free conditions), and should avoid chromatographic isolations.[2] Usually, reactions included in such a group have a large thermodynamic driving force and involve the formation of a carbon–heteroatom bond. A few synthetically useful reactions fulfil such criteria: opening of strained rings such as epoxides and aziridines,[4] oxygen additions to carbon–carbon double bonds (epoxidations),[5] and 1,3-dipolar[6] and Diels–Alder[7] cycloadditions.

Click chemistry has been especially successful in the preparation of 1,2,3-triazoles by a 1,3-dipolar cycloaddition between azides and alkynes. The original reaction, known as the Huisgen cyclization, involved a thermal treatment of both reagents and afforded the corresponding triazoles with a complete lack of regioselectivity, as a 1:1 mixture of the 4- and 5-substituted derivatives.[6]

Nevertheless, the discovery that this reaction can be efficiently catalyzed with copper(I) salts to give exclusively the 4-substituted regioisomer resulted in an excellent procedure for the preparation of such heterocycles.[2]

The interesting properties of the triazole scaffold, not only from a synthetic point of view, but also in the context of biological and pharmacological applications, led to a significant increase in click chemistry research. Furthermore, the triazole ring was shown to be compatible with a plethora of functional groups, as well as exhibiting good stability under several reaction conditions. Thus, much effort has been devoted to the preparation of triazole-containing peptides, oligosaccharides,[8,9] and natural product analogues,[10,11] and an enormous development in click chemistry has been achieved in the last few years. The preparation of dendrimers,[12,13] polymeric materials,[14,15] liquid crystals[16] and potential pharmacophores[17] has been achieved through this revolutionary concept. Not surprisingly, this modified triazole synthesis has become the paradigm of click chemistry in modern literature and both terms are now invariably associated, even though Sharpless' seminal ideas offer promising perspectives on other synthetic platforms.

In this review, we cover recent applications of click chemistry, particularly those involving the opening or formation of strained rings, as well as 1,2,3-triazole formation,

while highlighting their connection to macromolecular chemistry and drug-candidate searches.

2 *Click* Synthesis of Epoxides and Aziridines

Epoxides and aziridines are highly valuable intermediates in organic synthesis,[18–22] obtained mainly via oxidative processes from olefins.[23–26] The reaction of such intermediates with nucleophiles in a selective fashion is a typical example of a click chemistry reaction.[2]

α,β-Unsaturated acids and amides also undergo aminohydroxylation and dihydroxylation processes in a *click* fashion (good yields and extremely easy isolation) to afford the corresponding vicinal aminoalcohols or diols, respectively.[2,27]

A *click* example for the preparation of aziridines starting from olefins was reported by Siu and Yudin (Scheme 1).[5] Thus, a series of aziridines were prepared by an electro-

chemical process, in moderate to good yields, and the reaction could be scaled up to a multigram level. With this green procedure, hazardous oxidants and metal additives are avoided.[5]

R = phthalimido
R^1–R^4 = alkyl, aryl: cyclohexene, cyclohexenone, cyclooctene, *trans*-methyl cinnamate, etc.
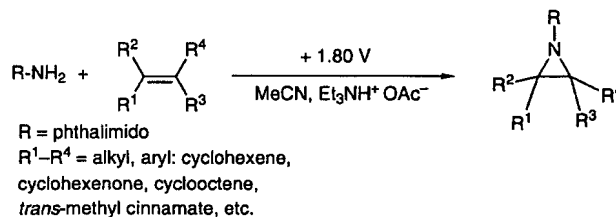
Scheme 1

Voronkov et al.[28] accomplished the practical and scaleable synthesis of isomeric limonene aziridines starting from commercially available limonene oxides, compounds of interest as key chiral intermediates. A 1:1 mix-

Biographical Sketches

María Victoria Gil was born in 1973 in Badajoz, Spain. She studied chemistry at the University of Extremadura and completed her degree in 1996. She received her PhD from the same university in 2000 under the supervision of Professors Emilio Román and José Antonio Serrano. She then pursued studies with Professor Roberto Ballini at the University of Camerino (Italy). In 2002, she was appointed as research assistant in the organic chemistry department at the University of Extremadura and in 2006 was promoted to assistant professor. Her research interests include asymmetric synthesis in the field of nitro compounds.

María José Arévalo was born in 1975 in Badajoz, Spain. She obtained her degree in chemistry at the University of Extremadura in 1998. After that, she joined the Department of Organic Chemistry at the same university, where she was involved in the study of the reactivity of 1,3-thiazolium-4-olate system derived from carbohydrates, getting her PhD in 2002 under the supervision of Drs. Martín Ávalos and José Luis Jiménez. She then obtained a postdoctoral fellowship to join the group of Prof. Albert Padwa at Emory University for two years, studying the 1,3-dipolar cycloaddition of nitrones. Currently, she is interested in isocyanide multicomponent chemistry and she is developing a project in that field at the Scientific Park of Barcelona.
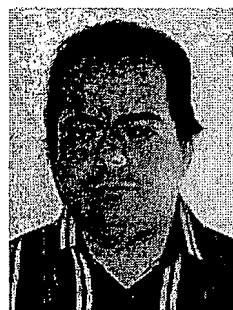
Óscar López was born in Seville (Spain) in 1976 and received his Diploma in chemistry from the University of Seville in 1999. He completed his PhD in the same university in 2003, concerning new synthetic approaches for the preparation of sugar-derived ureas, thioreas and selenoureas. In March 2004, he was appointed as lecturer in Environmental Organic Chemistry at the University of Huelva, Spain. In June 2004, he was appointed as lecturer in Organic Chemistry at the University of Seville, in the Faculty of Chemistry, where he is currently an assistant professor. From 2005–2006 he carried out a postdoctoral stay in the Department of Chemistry of Aarhus University (Denmark) in the group of Professor Mikael Bols; during this period, his research was focused on the preparation of cyclodextrin-based artificial enzymes and new iminosugars as glycosidase inhibitors.

ture of commercially available limonene oxides **1** was subjected to sodium azide mediated stereoselective opening of the epoxide ring, affording the corresponding isomeric α-azido hydroxy derivatives **2** and **3** (Scheme 2). Treatment of the mixture with triphenylphosphine at room temperature via a pseudo-Staudinger reaction[29] allowed a kinetic resolution, as the tertiary azide underwent a much slower reaction. Acid–base extraction followed by vacuum distillation afforded pure **4**; harsher conditions (refluxing 1,4-dioxane) gave access to the diastereoisomeric azide **5**.[28]
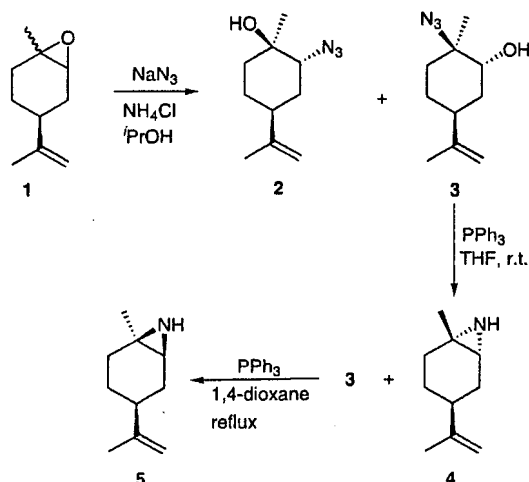
Scheme 2

A different approach for the preparation of aziridines was reported by Fioravanti and co-workers,[30] using, as starting materials, the functionalized enones **6**, which were either commercially available or readily synthesized (Scheme 3). The aziridination step was carried out by treatment of the enones with arylsulfonyloxycarbamates in the presence of an inorganic base such as calcium oxide. These reactions proceeded in high yield, with high purity, and the results were not dependent upon the substituents of the aminating reagent; furthermore, aziridines **7** could be used without chromatographic purification.
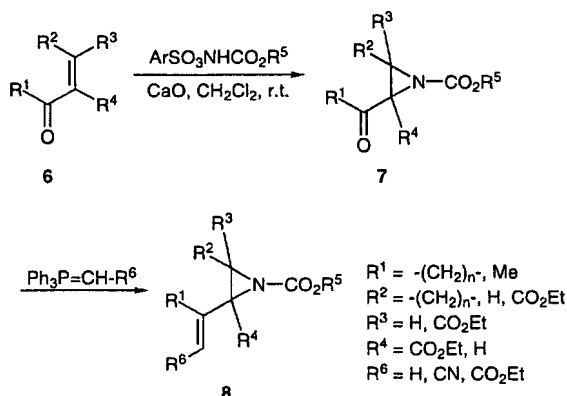
Scheme 3

The prepared aziridines were then transformed into the corresponding alkenyl aziridines **8** via a Wittig reaction with high conversion and excellent E-stereoselectivity (up to 99%).[30]

3 Nucleophilic Opening of Strained Rings: Epoxides and Aziridines

The stereoselective opening of strained rings, such as epoxides, aziridines and aziridinium ions, is a valuable synthetic tool for the preparation of more complex derivatives. In this context, Wong's group reported a versatile methodology for the rapid discovery of enzymatic inhibitors, where the key step is an amine-mediated opening of epoxides in an aqueous medium.[31]

The epoxide-containing core, **12**, was prepared from commercially available epoxide **9** as depicted in Scheme 4. The epoxide moiety in the starting material underwent ring opening upon treatment with p-thiocresol in basic medium to afford derivative **10**; attack of the thiolate reagent took place selectively at the less-hindered carbon of the epoxide moiety. Next, the Boc protective group was removed under acidic conditions and the transient free amino group was converted into the tetrahydrofuran-3-yl carbamate **11**. Finally, the epoxide moiety was regenerated by use of trimethyloxonium tetrafluoroborate, followed by treatment with potassium carbonate to give **12**.[31]

Epoxide **12** was subjected to ring opening with a series of primary amines to afford a small library of derivatives **13** that were screened as enzymatic inhibitors against HIV-1 PR and mutant V82A proteases. Compounds bearing either 2-amino-p-cresol or 4-phenoxyanilino moieties showed good inhibitory properties against both wild and mutant proteases.

Bulkier substituents in the amino residue were prepared starting from commercially available **9** and following a modified synthetic approach (Scheme 5),[31] where one of the key steps was again an amine-mediated epoxide opening. In this case, the first step was the epoxide opening of **9** using a primary amine, followed by tosylation of the amino residue of **14** with p-methoxybenzenesulfonyl chloride. Removal of the protective group of **15**, followed by introduction of the tetrahydrofuranyl moiety as indicated in Scheme 4, afforded derivatives **16** (Scheme 5), which exhibited potent viral protease inhibition in the nanomolar range (Table 1).

Fringuelli's group has carried out an exhaustive study of the epoxide opening with numerous nucleophiles and reaction conditions, such as pH and Lewis acid catalysts. Thus, aminolysis,[32] iodolysis, bromolysis,[33,34] azidolysis[33,35,36] and thiolysis[37–42] of this ring have all been reported by these authors, with the background of these reactions fulfilling the click chemistry criteria.[2] In particular, the thiolysis of epoxides was accomplished in a water medium.[40,42]

Scheme 4

Scheme 5

diol, a side-product (roughly 5%) obtained by attack of the hydroxide nucleophile, is water soluble, whereas most targeted β-hydroxy aryl sulfides **19** can be recovered by simple filtration in good yields (Table 2).

Table 1 K_i Values of Compounds **16** against HIV Proteases

Compound	R	HIV-1 PR	V82A
16a		3 nM	8 nM
16b		8 nM	13 nM
16c		3 nM	8 nM
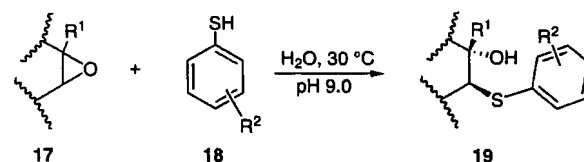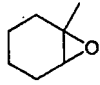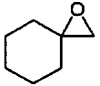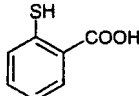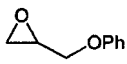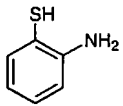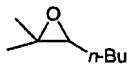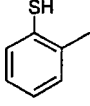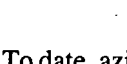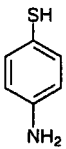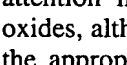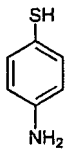
Scheme 6

Bandini et al. described[43] an indium(III) bromide catalyzed addition of indoles to chiral aromatic epoxides **20** (Scheme 7). The reaction proceeded via a purely S_N2 pathway and the mild conditions allowed for the isolation of the corresponding 2-aryl-2-(3′-indolyl)ethan-1-ols such as **22** without significant racemization. Furthermore, this procedure took place regioselectively and with high enantioselectivity (up to 99%).

For example, an easy and environmentally friendly thiolysis of substituted epoxides **17** by aromatic thiols **18** was carried out[40] in basic medium and in the absence of a metal catalyst (Scheme 6). Under these conditions, the reaction took place in a regioselective fashion on the less hindered carbon of the epoxide; furthermore, the vicinal
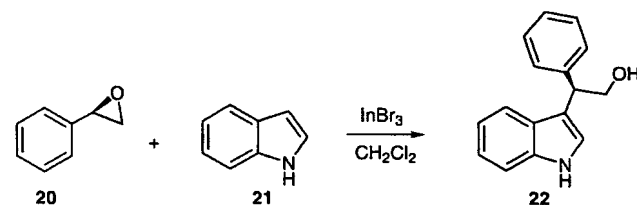
Scheme 7

Table 2

Epoxide **17**	Aryl sulfide **18**	Yield (%)
(1-methyl-7-oxabicyclo epoxide structure)	SH-phenyl	90
(same epoxide)	SH-benzene with COOH (2-mercaptobenzoic acid)	90
(same epoxide)	SH-benzene with NH₂ (2-aminothiophenol)	90
(same epoxide)	SH-toluene (2-methylthiophenol)	90
(1-oxaspiro epoxide structure)	SH-phenyl	95
(glycidyl phenyl ether, OPh epoxide)	SH-benzene with NH₂ (4-aminothiophenol)	94
(n-Bu epoxide structure)	SH-benzene with NH₂ (4-aminothiophenol)	95

Scheme 8

To date, aziridines and aziridinium ions have received less attention in organic synthesis than their isomeric epoxides, although this situation seems be changing. Under the appropriate reaction conditions, these intermediates can afford vicinal diamino moieties, a structural motif of particular relevance.[44]

Usually, the opening of aziridines that bear no electron-withdrawing group on the nitrogen atom involves the use of acidic activating agents.[44] Recently, Krasnova and Yudin reported the preparation of novel chelating agents[45] where the key step was the opening of an aziridine ring with nitrogen nucleophiles such as hydrazine or a tetrazole derivative (Scheme 8). Racemic aziridine derivative **23** (7-azabiciclo[4.1.0]heptane) was prepared from the corresponding epoxide in a manner similar to that described in Scheme 2, in a two-step process: opening of the epoxide with sodium azide was followed by ring closure to the aziridine motif by treatment with triphenylphosphine.

Compound **23** was subjected to opening using two different nucleophiles. Treatment with hydrazine afforded transient **24** that was used for the next step without further purification;[45] reaction with pentan-2,4-dione in refluxing
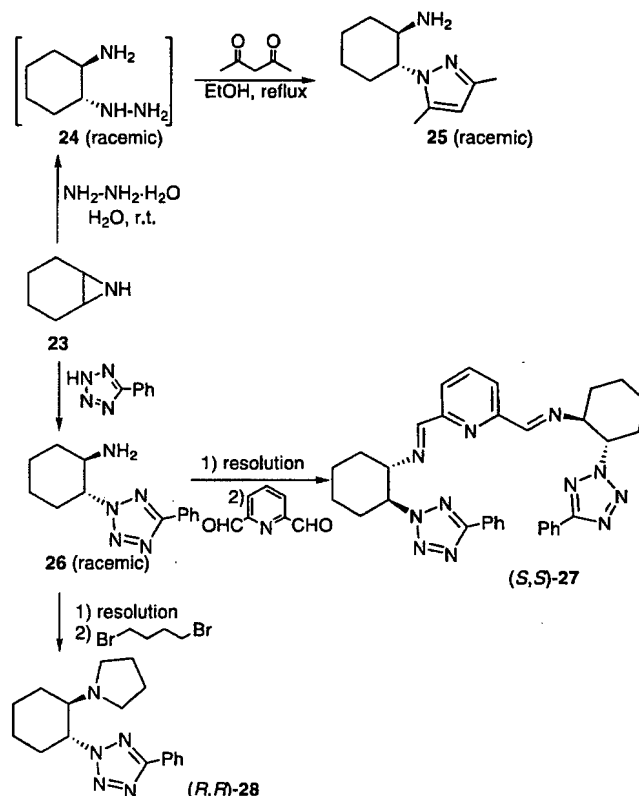
ethanol afforded pyrazole derivative **25**. Resolution of this compound was carried out using (S)-mandelic and (S)-camphorsulfonic acids, and enantiomerically pure **25** could be condensed with different aldehydes; however, the corresponding ligands showed limited stability.

When the opening of aziridine **23** was carried out with 5-phenyl-2H-tetrazole, derivative **26** was obtained as a racemic mixture; enantiomerically pure forms of **26** could be isolated upon resolution with (S)-mandelic and (S)-camphorsulfonic acids. Condensation of the S,S enantiomer with 2,6-diformyl pyridine afforded the dimeric ligand **27** (Scheme 8), which was complexed with copper salts and showed high diastereoselectivity in the styrene cyclopropanation reaction. Reaction of (R,R)-**26** with 1,4-dibromopropane gave access to tertiary amine **28** in moderate yield.

Sharpless and co-workers have exploited[44,46,47] the *click* opening of aziridinium ions with different nucleophiles. Unlike aziridine moieties, these useful synthetic intermediates can be opened at moderate temperatures, and at neutral and even basic pH. For example, pyrazolidin-3-ones were prepared using a spontaneous aziridinium ring opening as the key step.[46]

Reaction of the oxirane-containing ester **29** with secondary amines afforded derivatives **30** (major) and **31** (minor), that could be separated by crystallization.[46] However, separation of the regioisomers is not necessary, as mesylation of the free hydroxyl group in the crude reaction leads, in both cases, to the aziridinium ion **32**. The

latter undergoes a spontaneous nucleophilic displacement by chloride ion to afford the building block **33** (Scheme 9), which is a valuable starting material that has been used in the preparation of pyrazolidin-3-ones,[46] β-lactams or benzodiazepinones[47] (Figure 1).

Scheme 9

NR_2 = morpholino, diallylamino
R^1 = t-Bu, CH_2C=CH, n-Bu
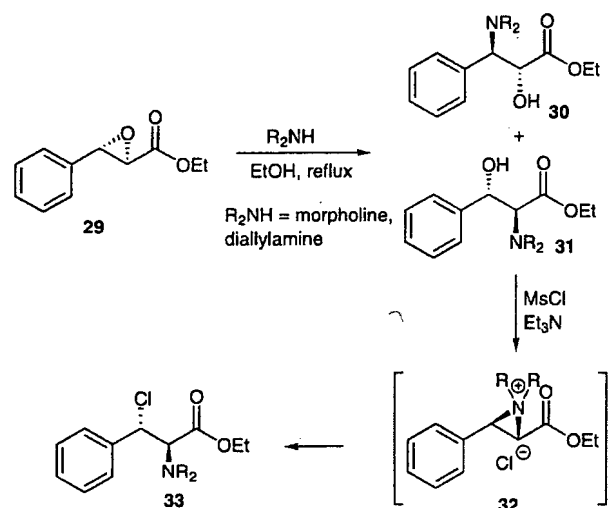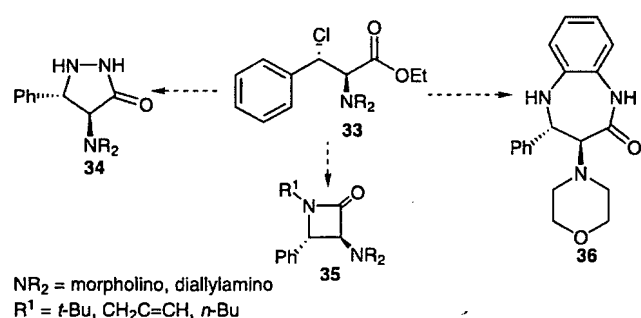
Figure 1

4 Huisgen 1,3-Dipolar Cycloadditions

The 1,3-dipolar cycloaddition that involves azido and alkyne (terminal or internal) derivatives is known as the Huisgen cyclization, and gives access to 1,2,3-triazoles as a mixture of 1,4- and 1,5-regioisomers (Scheme 10).[48] The lack of selectivity can be explained by the similarity in activation energies for both processes.[49]

Such intrinsic features of the Huisgen cycloaddition make it unsuitable for being considered as a *click* reaction. Nevertheless, the observation that copper(I) salts promote

1,4-regioisomer   1,5-regioisomer
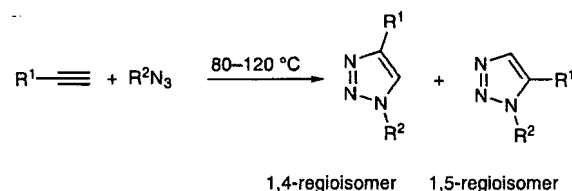
Scheme 10

faster (up to 10^7 times) and regiospecific couplings between terminal alkynes and azides allowed for the rapid development of this reaction; these results were reported independently by the groups of Sharpless[50] and Meldal.[3] Moreover, the cycloaddition is usually carried out at room temperature in aqueous media, and is compatible with most common functional groups present in the molecules.

Usually, the source of copper(I) is the reduction of copper(II) sulfate in the presence of sodium ascorbate,[50] although some other conditions have been reported, such as copper(I) salts,[3] copper(I) complexes,[51] and copper(I)-stabilized derivatives.[52]

The accepted mechanism,[49,50,53] shown in Scheme 11, involves the initial formation of a π complex between a terminal alkyne and copper(I), thereby lowering the pK_a value of the alkyne. Under these conditions, the acetylene derivative is acidic enough to be deprotonated in aqueous medium.
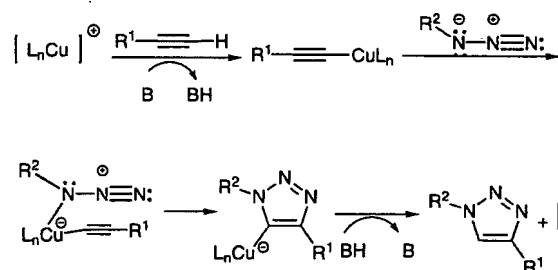
Scheme 11

This copper(I) acetylide evolves to a copper(I) adduct upon attack of the organic azide, and then undergoes an intramolecular cyclization to give a copper-containing 1,2,3-triazole. Final protonation regenerates the catalyst and gives the 4-substituted 1,2,3-triazole derivative. Overall, the postulated mechanism is a stepwise, not a concerted, pathway.

Recently, Sharpless and co-workers demonstrated[54] that ruthenium complexes such as $Cp^*RuCl(PPh_3)_2$ catalyzed the Huisgen 1,3-dipolar cycloaddition; interestingly, the observed regioselectivity was reversed when compared with related copper(I) catalysis. A tentative mechanism suggested by the same authors (Scheme 12)[54] involves a six-membered ruthenacycle intermediate, which undergoes reductive elimination, affording a 5-substituted triazole derivative. Unlike in the case of the copper(I)-mediated reaction, both terminal and internal alkynes can participate, which would mean that the formation of ruthenium acetylides can be discarded as a hypothesis.

Weinreb's group reported the preparation of 5-substituted 1,2,3-triazoles **40** by reaction of β-tosylethylazide (**38**) with alkynes in the presence of ruthenium catalysts in refluxing benzene (Scheme 13).[55] When the standard copper(I)-catalyzed protocol was used, it was found that reaction of β-tosylethylazide with terminal alkynes led to the expected 4-substituted 1,2,3-triazoles.[55]
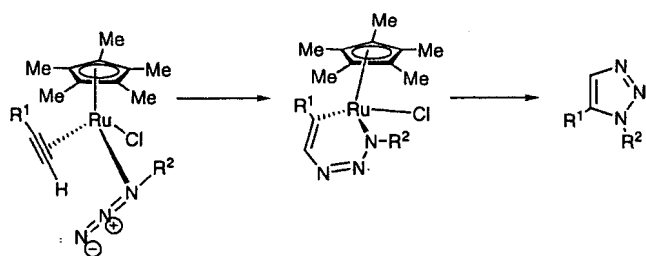
Scheme 12
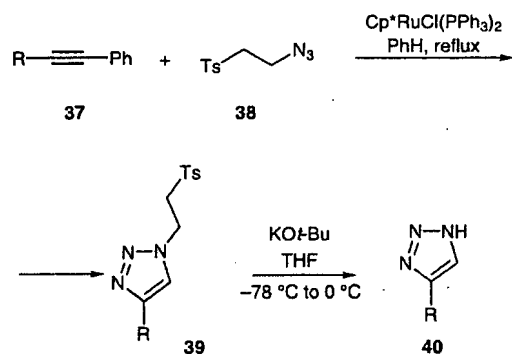
Scheme 13

R = Ph, 4-MeC$_6$H$_4$, Bu, Hex, PhOCH$_2$, 4-MeOC$_6$H$_4$

As an attempt at improving the catalysis for 1,3-dipolar cycloaddition reactions between azides and terminal alkynes, active and stable nanometric copper clusters have been proved to be efficient ligand-free catalysts.[56] These clusters are prepared with a narrow cluster-size distribution as a stable solution by reducing copper(I) chloride in solution with tetraoctylammonium formate.

To prove the efficiency of this new catalyst, the kinetics of a model reaction between benzyl azide and prop-2-yn-1-ol catalyzed by copper shavings, copper powder, copper nanoclusters, and copper(II) sulfate/ascorbate were monitored. Copper nanocluster catalysis displayed the highest activity of the four systems tested, affording 100% conversion after 18 hours, and this result was in good agreement with the increased surface area of the nanoclusters. The specific surface area of the clusters and the powder was 168 m^2/g and 0.15 m^2/g, respectively.[56]

An extended application of this reaction to the synthesis of electronic and optoelectronic materials is the coupling of azides with ruthenium-containing alkynyl biosensors.[57] The corresponding di(ruthenium)-containing *click* product is obtained in excellent yields.

With the aim of exploring the feasibility of the click chemistry with ynamides and azides, coupling between *N*-benzyl-*N*-tosylynamide and *N*-Boc-2-azidoethylamine was also tested. This approach was broadened to potentially bioactive compounds, and carbohydrates and amino acids containing azido groups were *clicked* with a series of ynamides to yield the corresponding cycloadducts.[58] Nevertheless, when the *click* Huisgen cycloaddition was attempted between copper(I) acetylides and sulfonyl

azides, *N*-acylsulfonamides were isolated in aqueous medium,[59] whereas amidines were obtained in the presence of secondary amines.[60]

Another unexpected reaction was reported by Whiting and Fokin to take place between phenylacetylene and *p*-toluenesulfonyl azide.[61] Despite obtaining the corresponding 1-sulfonyl-4-phenyl-1,2,3-triazole, the only isolated product was a cyclobutene derivative. Furthermore, when the reaction was accomplished in the presence of *N*-benzylideneaniline, an azetidinimine was obtained, together with a small amount of the 1,4-disubstituted triazole.[61]

A catalyst-free alternative to carry out the covalent modification of biomolecules in living systems consists of a strain-promoted [3+2] cycloaddition. Following this methodology, Agard et al. reported the synthesis of triazole compounds by reaction of azides and cyclooctyne derivatives under physiological conditions.[62]

The *click* Huisgen 1,3-dipolar cycloaddition has also been extended to the synthesis of tetrazoles when organic azides and nitriles are used. Upon simple heating of neat *p*-toluenesulfonyl or acyl cyanides **42** and **43** with one equivalent of various unhindered azides **41**, quantitative conversion to the corresponding 1-alkyl-tetrazole derivatives **44** and **45** was achieved (Scheme 14).[63] The reaction was run neat, there were no side products, yields were quite high and isolation was simple. In all cases, the reaction was highly regioselective and only one isomer was observed. Unfortunately, bulky azides and aryl azides are not appropriate for this reaction, and only certain highly electron-deficient nitriles are good enough as dipolarophiles to engage organic azides in an intermolecular fashion.[63]
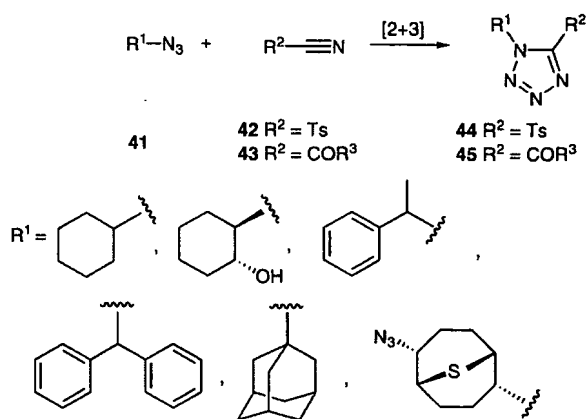
Scheme 14

4.1 Supramolecular Click Chemistry

4.1.1 Dendrimers

Dendrimeric materials are extremely attractive candidates for a variety of surface-active applications, such as multivalent binding sites for interaction with biological receptors and cell surfaces in the construction of targeted drug delivery systems.[64–66] Dendrimers contain three distinct

structural parts, namely the core, end-groups, and branched units connecting core and periphery. These materials have been synthesized by methodologies based on both convergent and divergent routes.[67,68] The convergent approach, introduced by Fréchet's group,[69] introduces the core scaffold in the final step. This method, on the one hand, enables the introduction of the desired functionalities in the structural building blocks,[68] and on the other hand, implies fewer coupling reactions, more exact macromolecular architectures, greater monodispersity, and greater control over the placement of desired functionalities. In contrast, the divergent approach allows for the formation of the dendrimeric structure by way of iterative additions to the core scaffold.

It happens that the Huisgen 1,3-dipolar cycloaddition is a simple and reliable procedure for the efficient synthesis of chemically differentiated dendrimers.[70–73] The methodology is characterized by 1,4-regiospecific 1,2,3-triazole formation, water tolerance, and acceptance of a wide range of functionalities.[74] There are three strategies for triazole dendrimers: coupling reaction between a dendron azide and a dendron alkyne, between a dendron azide and polyalkynes or between a dendron alkyne and polyazides.

In this context, polyvalent dendrimeric peptides that may be useful in the preparation of synthetic vaccines have been accessed via an efficient Huisgen 1,3-dipolar cycloaddition. Thus, Rijkers and co-workers accomplished the microwave-promoted coupling of polyvalent alkynes and azides in the presence of copper(II) and sodium ascorbate (Scheme 15).[15] Using these conditions, the tetravalent dendron 46 was coupled with the azido-containing amide 47 to afford derivative 48. Dendrimeric cycloadducts were isolated in moderate to good yields (50–95%).

Sharpless, Hawker, and co-workers[75] used the Huisgen 1,3-dipolar cycloaddition of dendrons containing azide (49) and alkyne (50) moieties to obtain third-generation dendrimer 51 as a biocompatible building block (Scheme 16). It is noteworthy that, as indicated above, Huisgen cycloaddition reactions are compatible with a plethora of functional groups; for instance, as depicted in Scheme 4, the reaction is not hindered by to the presence of free hydroxyl groups and ester moieties.

The introduction of mannose and coumarin units to the periphery allowed for the preparation of unsymmetrical dendrimers that proved to be efficient agents for the inhibition of hemagglutination.[75]

Propargyl-functionalized Fréchet-type dendrons 52, that is, poly(benzyl ether) dendrons, have been applied to the convergent synthesis of dendrimers using tripodal azide core 53 or azide-focal-point-functionalized Fréchet-type dendrons 55.[76] Reactions were carried out in aqueous N,N-dimethylformamide, under the standard copper-catalyzed Huisgen reaction conditions, and the corresponding symmetrical and unsymmetrical triazole-containing dendrimers were obtained in good yields (Scheme 17).[76]

Polyamidoamine-based dendrimers, so-called PAMAMs, represent a new class of macromolecular structures, and they are sometimes referred to as 'dense star' polymers.[77]
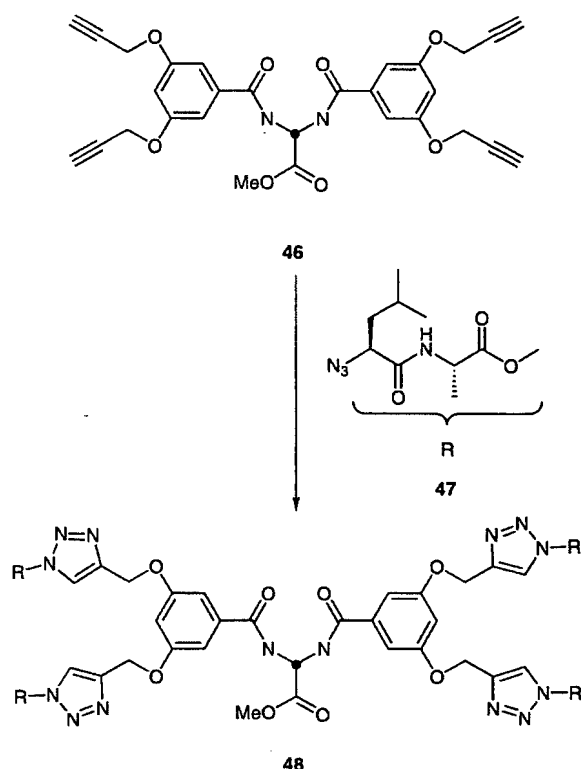
Scheme 15

A standard synthesis of these polymers is based on a divergent approach that involves a Michael addition of methyl acrylate and the amine core to afford the ester moiety, which is subjected to saponification and coupling with a diamine; subsequent iteration leads to the title compounds.[78] These polymers were recently found to solubilize water-insoluble drugs and to promote their transport through biological membranes.[79,80] Furthermore, the presence of a large number of exo-functional groups might allow their use as molecular biosensors.[81] A high-yielding synthesis of this interesting family of dendrimers was recently achieved by Lee et al.,[12,82] in which the azido moieties were efficiently connected to the bis(alkyne) core unit 58 via a copper(I)-catalyzed Huisgen reaction to furnish PAMAM dendrimers 59 in 99% yield (Scheme 18).

Introduction of carbohydrate units to dendrimeric structures might lead to a glycoconjugate that could participate in recognition processes with biological receptors.[83] However, synthetic routes described so far for the preparation of glycodendrimes[84,85] usually involve long syntheses that afford unprotected coupled sugar scaffolds. In this context, Riguera's group accomplished[86] the anchoring of a series of carbohydrate-derived acetylenes into azido-terminated dendrimers through regioselective 1,2,3-triazole formation (Scheme 19); the reported dendrimers incorporated up to 27 monosaccharidic units (L-fucose, mannose, and lactose), with yields up to 92%. The inherent conditions of this reaction (environmentally friendly, regioselective, high-yielding) make this dendrimer derivatization a good candidate for the preparation of glycoconjugates of potential biological and pharmacological interest.
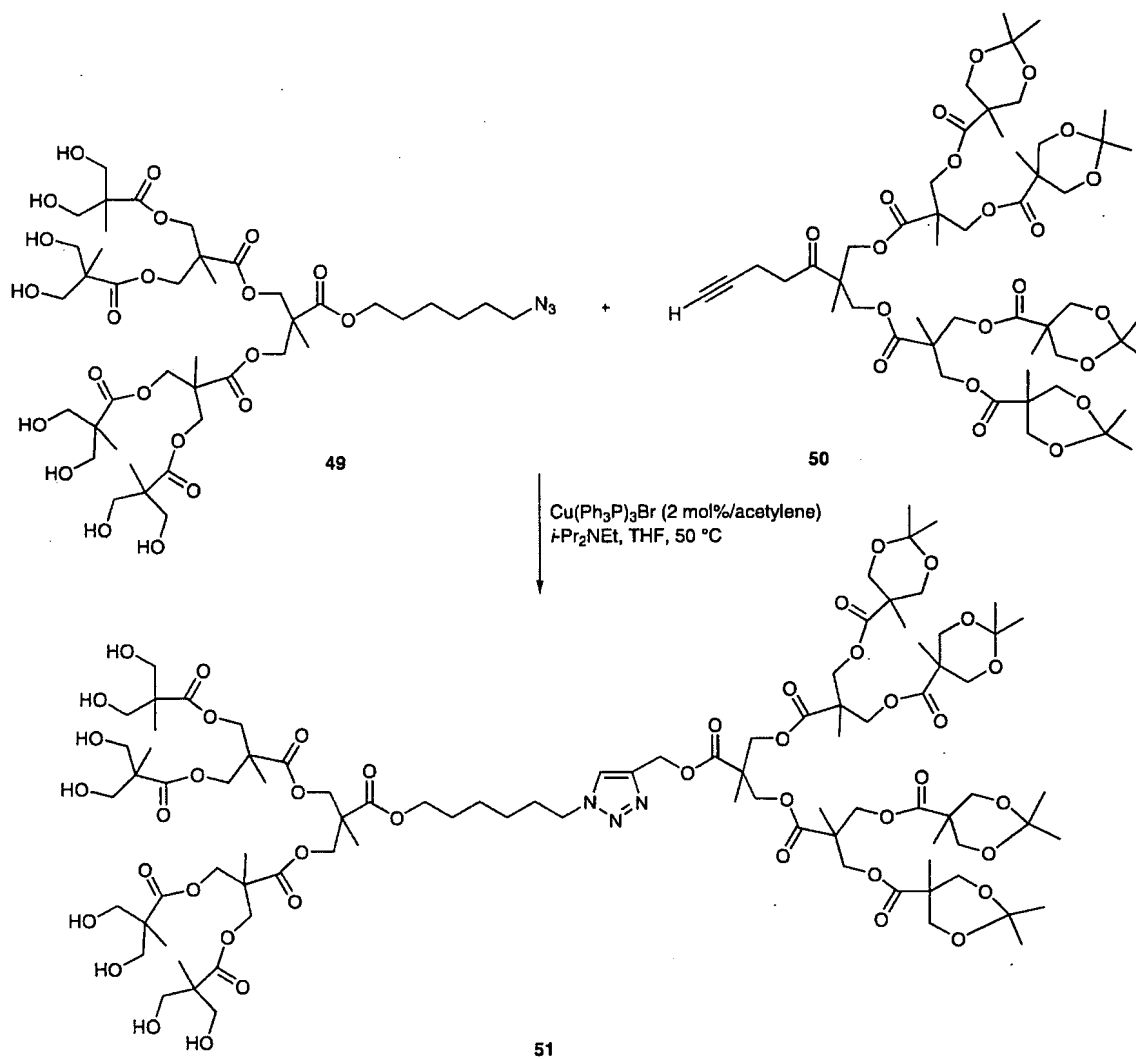
Scheme 16

Scheme 17

Scheme 18

60 α-L-fucoside   **61** α-D-mannoside   **62** β-D-lactoside
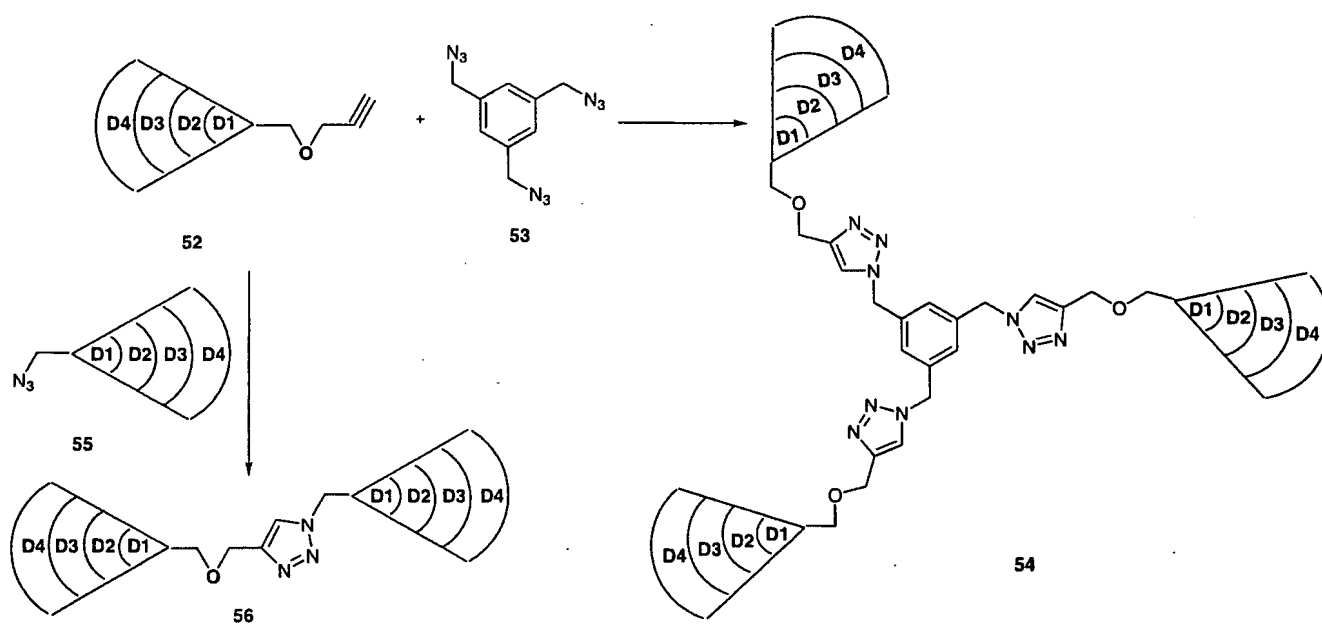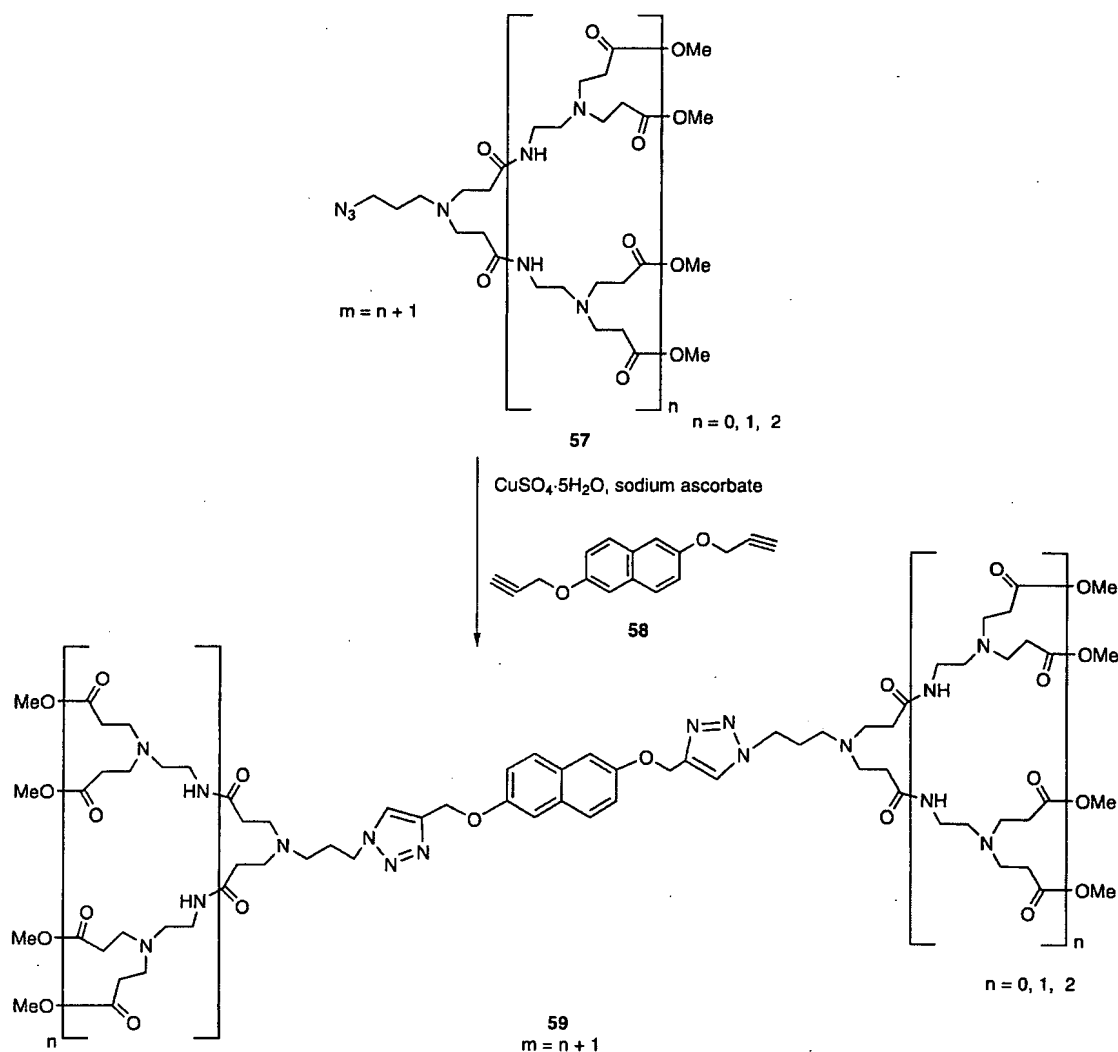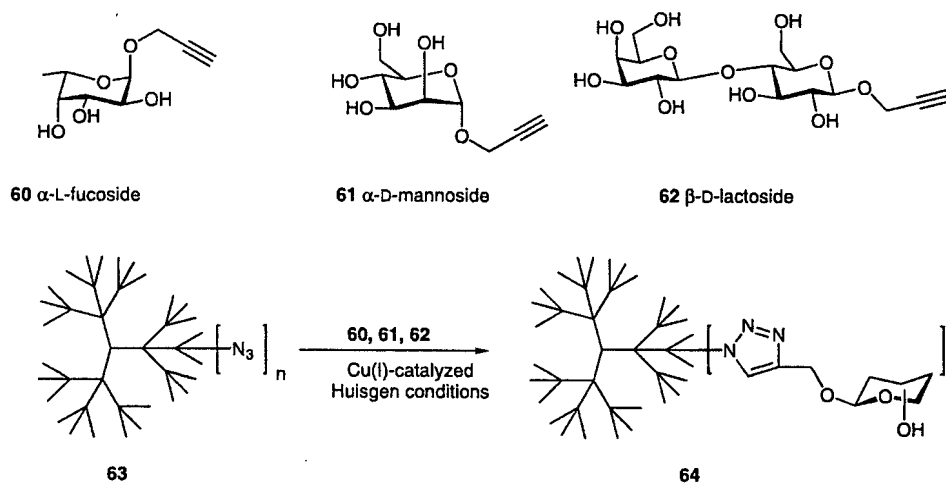
Scheme 19

Fréchet's group has also carried out the preparation of linear dendrimer-containing polymers using click chemistry.[13,87] The sizes and shapes of these polymers can be modulated; the existence of a linear architecture might improve their use in some nanoscale applications, such as catalysis or molecular electronics. Furthermore, sometimes the spherical shape of a classical dendrimer is not appropriate for some applications.[88,89] The approach used by

this group was to start from a linear polymer with pendant reactive groups (for example, alkyne moieties) and to carry out the coupling with an azido derivative.[13] A dendronized polymer was obtained from the first-generation dendron 65 and poly(vinyl acetylene) 66 in quantitative yield, as depicted in Scheme 20. The same kind of reaction was also reported to occur in almost quantitative yield (> 98%) for third-generation azide-containing dendrons.
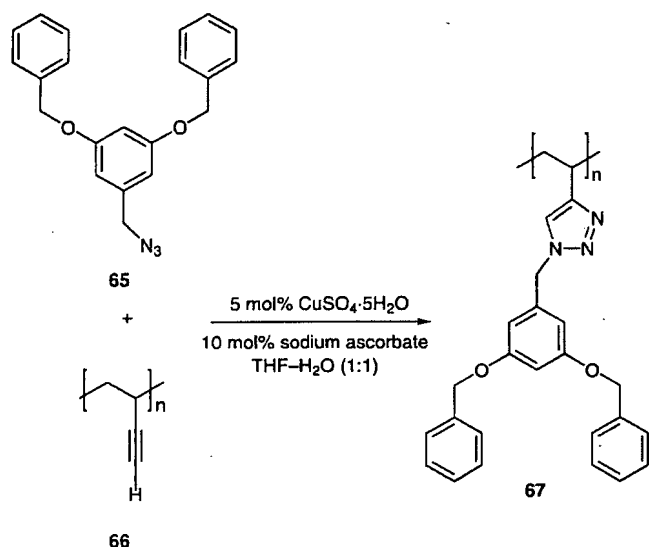
Scheme 20

Scheme 21

4.1.2 Polymers

The control of the molecular structure, and particularly the chemical functionalities, of polymers is a pivotal task in modern polymer synthesis, because complex macromolecules are needed in the rapidly growing fields of nanotechnology[90–92] and nanobiotechnology.[93–95] In this context, click chemistry provides a powerful tool for the selective modification of copolymers, and the copper-catalyzed [2+3] Huisgen cycloaddition has provided access to triazole-containing derivatives.[96–99]

Carroll et al. reported the preparation of flavin-derived polymers[100] with potential analytical applicability, for example as potential sensors.[101] Nucleophilic displacement of the chlorine atoms in chloromethylstyrene copolymer 68 with sodium azide, followed by copper(I)-mediated Huisgen cycloaddition with alkyne derivative 70 afforded the flavin-functionalized polymer 71 (Scheme 21). It is remarkable that the triazole scaffold has a strong dipolar moment and subsequently can establish hydrogen bonds with hydrogen donors; at the same time, they provide some hydrophilicity in the system while being stable under biological conditions. These specifically flavin-functionalized polymers displayed reversible redox-modulated recognition with complementary 2,6-diamidopyridine (DAP)-derived units, the binding association between the polymer and DAP being quantified through the use of fluorescence spectroscopy.[100]
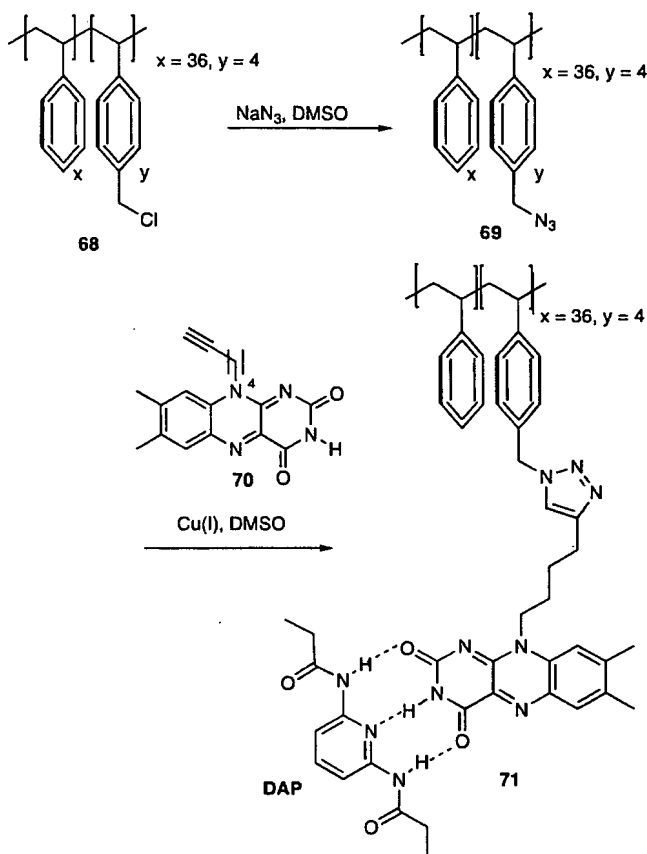
Polystyrene derivatives analogous to 68 have been converted into a triazolylmethyl acrylate (TMA) resin as a solid support for the efficient parallel synthesis of differently substituted tertiary amines.[102]

The nucleophilic substitution of a Merrifield-type resin with sodium azide allowed for the formation of azide-substituted polystyrene 72. The subsequent 1,3-dipolar cycloaddition with commercially available propargyl acrylate was conducted in N,N-dimethylformamide/ tetrahydrofuran in the presence of N,N-diisopropyl-ethylamine and a catalytic amount of copper(I) iodide, leading to the TMA resin 73 (Scheme 22). Then, N-benzyl-N-methylamine was added in N,N-dimethylformamide and the aminopropionate 74 was alkylated with 2,6-dichlorobenzyl bromide. Final cleavage to the TMA resin, induced by basic medium, led to the tertiary amine 75 in a 68% yield, with the progression of the cycloaddition being monitored by IR spectroscopic methods.[102]

Electrooptic polymers have been generated from the *click* Diels–Alder reaction between maleimide-containing non-linear optical (NLO) chromophores and polymers that possess pendant anthryl-containing diene moieties. This synthetic approach is very mild, versatile, quantitative, and free of ionic species and catalysts.[103]

The bromine chain-ends of a polystyrene prepared using atom-transfer radical polimerization were successfully transformed into various functional end-groups (ω-hydroxy, ω-carboxyl and ω-methylvinyl) by a two-step path-

propargyl acrylate
Cu(I)I, DIPEA

i. 2,6-dichlorobenzyl bromide
ii. Et₃N

72   73   74   75
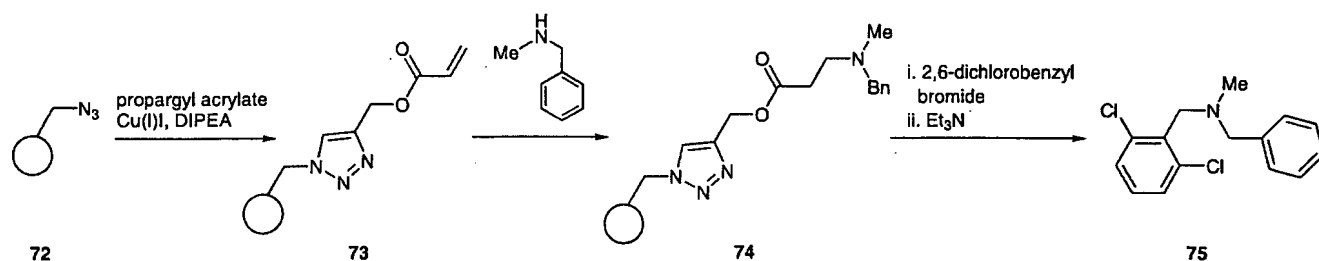
Scheme 22

way: first, nucleophilic substitution of the terminal bromine atom of **77** by an azide function, and then 1,3-dipolar cycloaddition of the terminal azide and functionalized alkynes to give derivatives **79–81** (Scheme 23). The 1,3-dipolar cycloaddition was catalyzed by the combination of copper(I) bromide and 4,4'-di(5-nonyl)-2,2'-bipyridine (a new catalytic system for this reaction which is well known in the atom-transfer radical polymerization field), and was quantitative after three hours.[104]

CuBr, PMDETA

NaN₃

76   77

78   79 R = CH₂OH
80 R = COOH
81 R = CH(Me)=CH₂
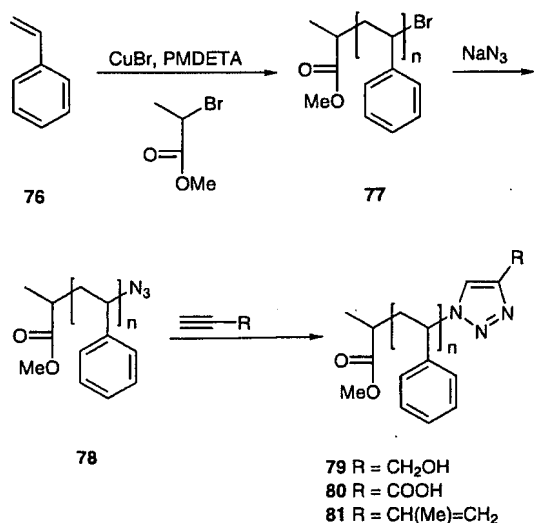
Scheme 23 PMDETA = *N,N,N',N'',N''*-pentamethyldiethylenetriamine

The derivatization of aliphatic polyesters by *click* reactions under very mild conditions allowed for the grafting of a wide range of functional groups onto poly(ε-caprolactone) ·without any protection or deprotection reactions.[105] α-Chloro-ε-caprolactam (**82**) was converted into azido-containing polyester **84** through two different synthetic routes (Scheme 24): by copolymerization with ε-caprolactam followed by displacement of the chlorine atom by sodium azide, or by initial displacement of the chlorine atom in the monomer to give **85** and final copolymerization of the latter with ε-caprolactam to afford **84**.

This azido-containing polymer was then subjected[105] to *click* cycloaddition with propargylic acid derivatives in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford grafted triazoles **86** under mild conditions and in short reaction times, with IR spectroscopic monitoring of reaction progression. Thus, this methodology provides a robust synthetic pathway for grafting a

plethora of functional groups onto a polymeric structure, either cationic, anionic or neutral, including labile lactic acid. The authors claim that the title polymers might be of interest in drug delivery and gene therapy.[105]

82   DSOP   83

NaN₃
DMF, 45 °C, 3 d

NaN₃
DMF, 45 °C, 3 d

85   DSOP

84

CuI, DBU,
THF, 35 °C

≡—CH₂R

86

R = OCOPh, Et₃N⁺Br⁻
NMe₂, Me₂N⁺PEOBr⁻
[PEO = poly(ethylene oxide)]
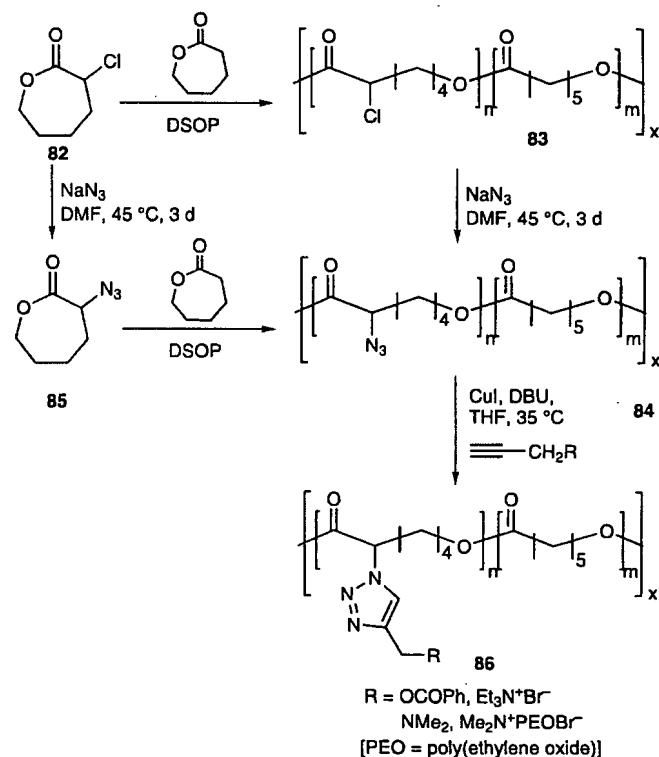
Scheme 24

Methyl triflate initiated polymerization of 2-(pent-4-ynyl)-2-oxazoline or copolymerization of this monomer with 2-methyl- or 2-ethyl-2-oxazoline afforded the corresponding poly(2-oxazolines) **87** and **88** (Scheme 25).[106] Subsequent copper-catalyzed Huisgen 1,3-dipolar cycloaddition with a series of azides afforded triazole derivatives **89–91** in quantitative yield; these compounds are interesting synthetic targets because they can be considered as prototypes for carrier systems in a new class of polymer-based therapeutics.[106]

Polymers made by a *click* post-modification of poly(*p*-phenylene ethynylene)s (PPEs) were compared[107] to those synthesized through a conventional approach. Wherever the structural repeat unit of the polymer was the same, the optical properties of these polymers in solution proved to be identical; nevertheless, thin-film optical and thermal

87 n = 45, m = 5
88 n = 35, m = 12

+ N$_3$CH$_2$R^2

CuSO$_4$·5H$_2$O
sodium ascorbate

89 n = 45, m = 5; R^2 = TMS
90 n = 45, m = 5; R^2 =
91 n = 35, m = 12 ; R^2 =
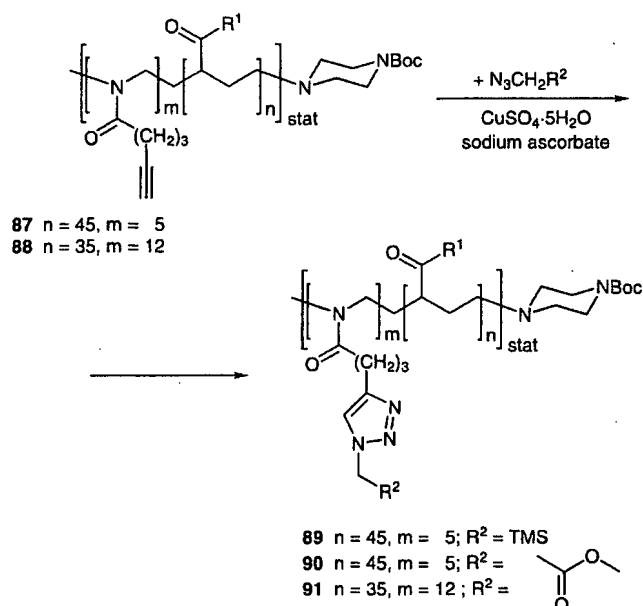
Scheme 25

properties differed as a result of varying degrees of polymerization and perhaps a different distribution of triazole regioisomers.

Another important tool for the synthesis and use of macromolecules in fields ranging from biomedical devices to nanotechnology is the development of selective synthetic approaches that are orthogonal to the diverse array of functional groups present in many polymeric systems. Thus, by combining copper(I)-catalyzed 1,3-dipolar cycloaddition with other families of chemical transformations, the synthesis of macromolecule **96** and the cascade methodology for the preparation of **102** were recently simultaneously reported by Hawker and co-workers.[108]

In the first case, an 8:1:1 terpolymer was prepared from styrene, p-(trimethylsilylacetylene)styrene, and 2-(trimethylsiloxyethyl)methacrylate using living-free-radical polymerization, and deprotection of both protecting groups with tetrabutylammonium fluoride (TBAF) gave difunctional macromolecule **95** [$M_n = 31900$ amu; PDI = 1.16 (PDI = polydispersity index)].[108] The acetylene and hydroxyl functionalities present in **95** afforded reactive sites that could couple simultaneously with both methyl 4-(azidomethyl)benzoate and the acetonide-protected bis-MPA [MPA = 2,2′-bis(hydroxymethyl)proprionic acid] anhydride in the presence of copper(I) and N,N-diisopropylethylamine (Scheme 26). The orthogonally functionalized macromolecule, **96** ($M_n = 39900$ amu;

92

93

94

a)
120 °C
b) TBAF, THF

95

N$_3$ ⋯ OMe
CuBr(PPh$_3$)$_2$

DIPEA

96
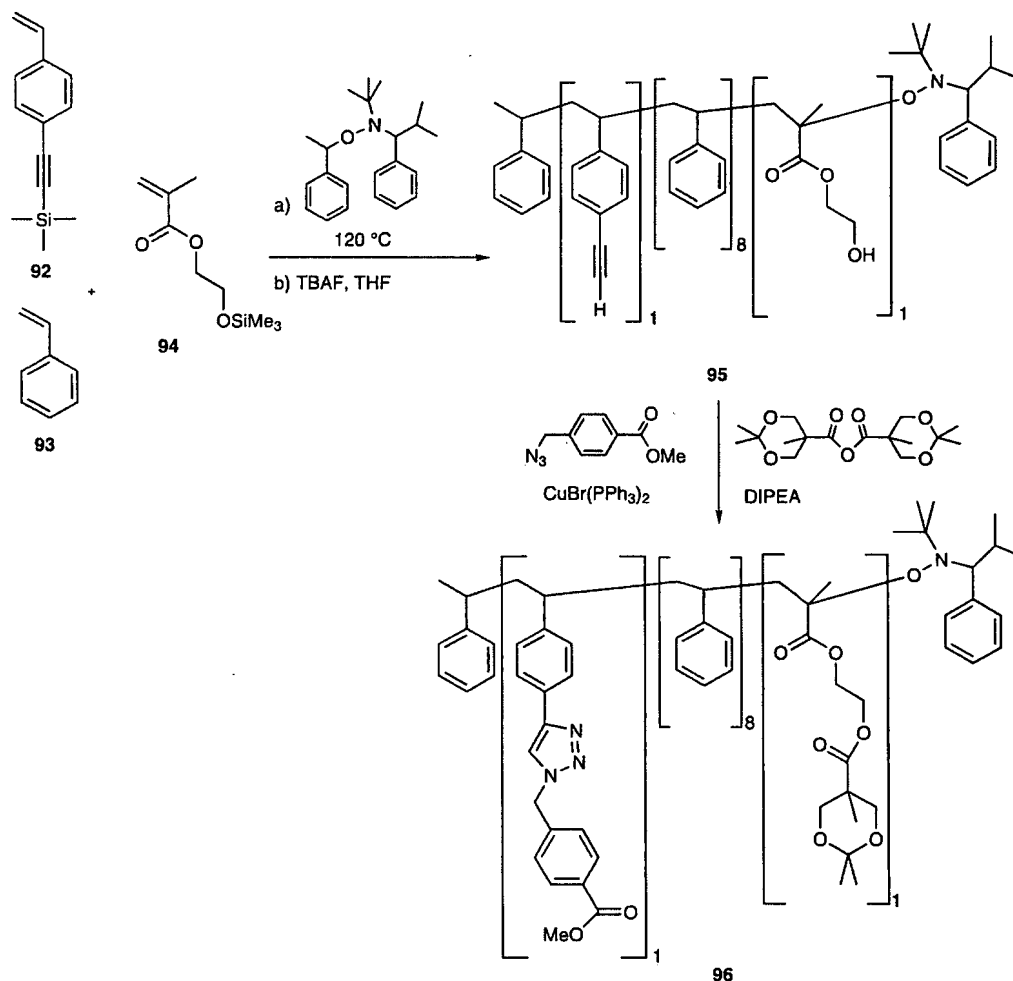
Scheme 26

PDI = 1.19), was obtained by transforming the terminal acetylene group into a methyl benzoate substituted triazole motif and by esterification of the hydroxyl group (Scheme 26).[108]

For the synthesis of **102** (Scheme 27),[108] to a solution of a 9:1 random copolymer of *tert*-butyl acrylate and *N*-acryloyloxy succinimide (**97**) in tetrahydrofuran were added propargylamine (**98**) and azo compound **99**. In this case, copper(I) acts as a catalyst for the *click* reaction between **98** and **99**, while the *N,N*-diisopropylethylamine catalyzes the amidation reaction of **97** with either **98** or the intermediary *click* adduct **101**. This sequence of reactions was monitored by GPC/HPLC and ¹H NMR spectroscopy,

which showed that all reactions reached completion after 16 hours at 50 °C and that the amidation chemistry and *click* reactions occurred at approximately the same rate.[108]

Conjugated polymers have been synthesized through the copper(I)-catalyzed 1,3-dipolar cycloaddition of azides and alkynes.[109] The novel polymers obtained, linked via 1,4-disubstituted 1,2,3-triazole units, have interesting luminescent properties. Fluorene-derived **103** reacted with derivatives **104** and **105** in a degassed tetrahydrofuran–acetonitrile solution. The reaction started with the addition of copper(II)/copper(0) and trisbenzyltriazolylamine as a ligand (Scheme 28). The polymerization reactions were stopped at both ends of the growing chain by the ad-

Scheme 27

Scheme 28

103a n = 5
103b n = 11

104 X = N
105 X = CH

Cu/Cu(OAc)₂

106 X = N, n = 5
107 X = N, n = 11
108 X = CH, n = 11
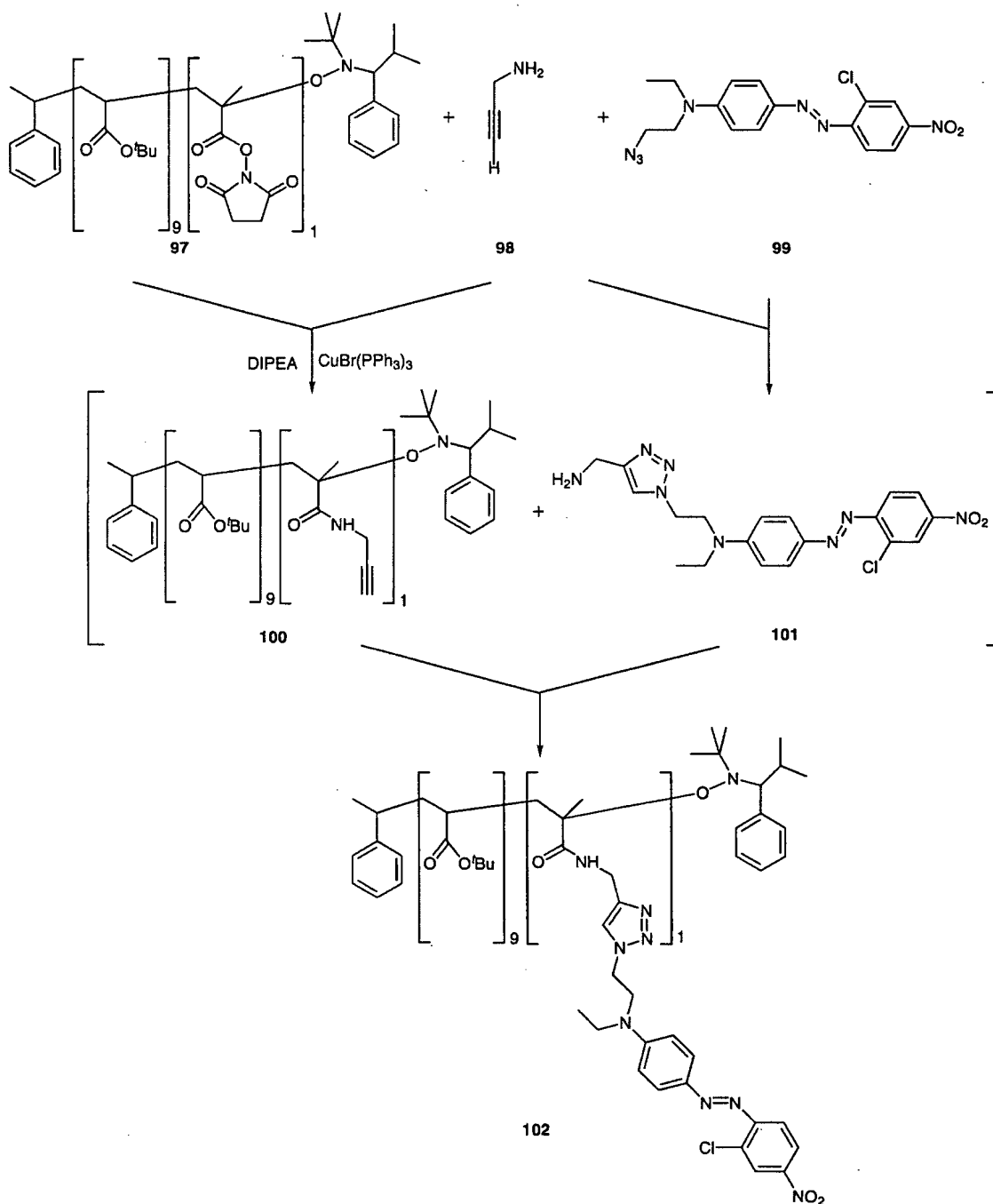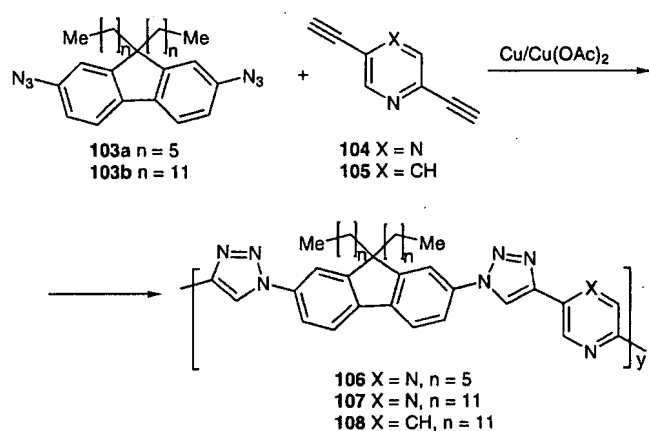
dition of excess azidobenzene as stopper, and after two hours, 2-ethynyl pyridine. The reactions were sufficiently fast to proceed at lower temperatures; when the reaction was accomplished at these low temperatures and under dilute conditions, polymers with high molecular mass and a more transparent appearance were obtained.[109]

Copper(I)-catalyzed reactions have also been used successfully in macromolecular chemistry for the preparation of stable and thermoreversible cross-linked organogels through coupling between alkynes and azide-containing organogelators in a 10:1 ratio (Scheme 29).[110] This method allows for the modification of some of the parent gel's properties, while maintaining the overall structure and the thermoreversibility.

gel

N₃~~~~~~N₃   Cuᴵ

cross-linked gel
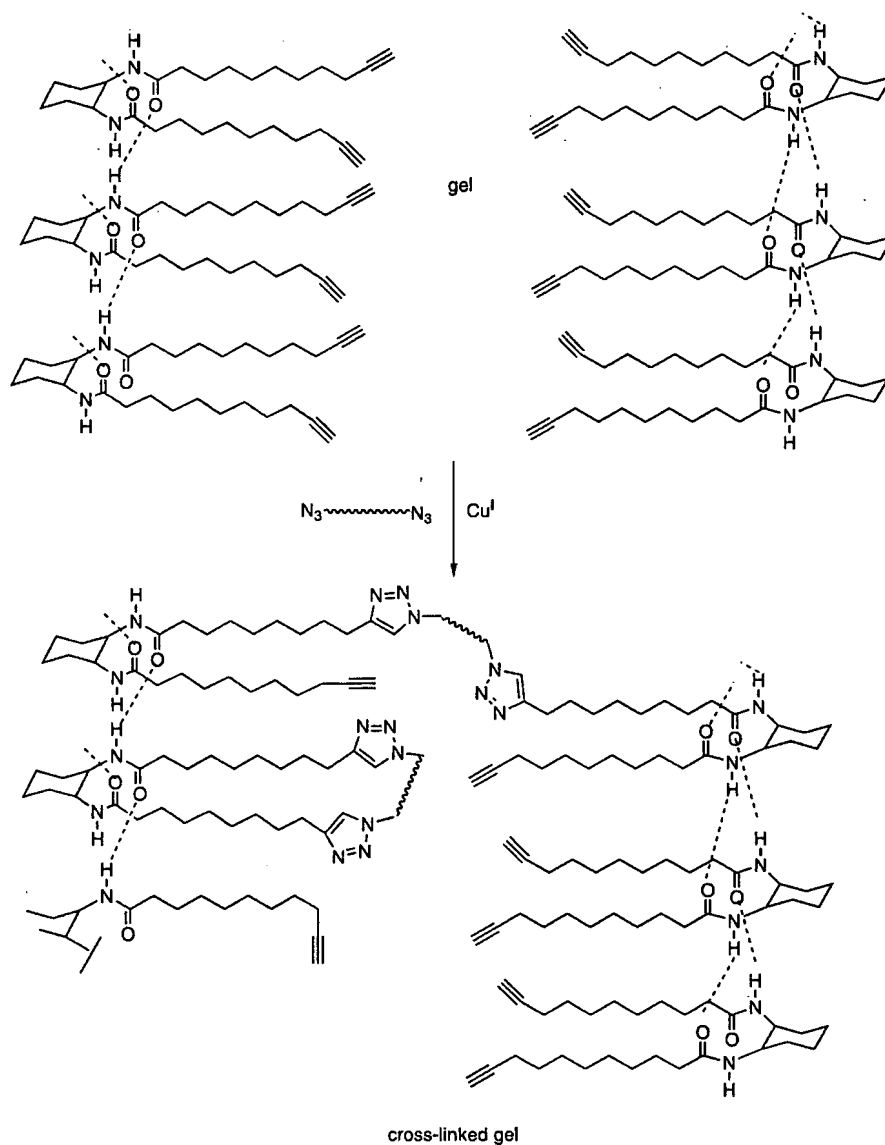
Scheme 29

Poly(vinyl alcohol)-based hydrogels have been prepared using this biocompatible copper(I)-catalyzed version of Huisgen [3+2] cycloaddition by two different cross-linking strategies.[111] Firstly, poly(ethylene glycol) diazide can be used as a cross-linker for the alkyne-functionalized poly(vinyl alcohol). Moreover, a multicomponent reaction involving two poly(vinyl alcohol)s plus amine-functionalized alkyne and azide substrates can also be considered (Scheme 30). Hydrogel properties are expected to be highly dependent upon the structure of the polymer components. The level of 100% gelation was not achieved,[111] probably because of incomplete 1,3-cycloaddition cross-linking reactions, or perhaps because of some side reactions that could occur as a result of the difficulty in achieving the proper mixing of reagents. The reaction appears to be quite fast, and the gels were formed essentially immediately upon addition of the copper catalyst to the polymer solution.
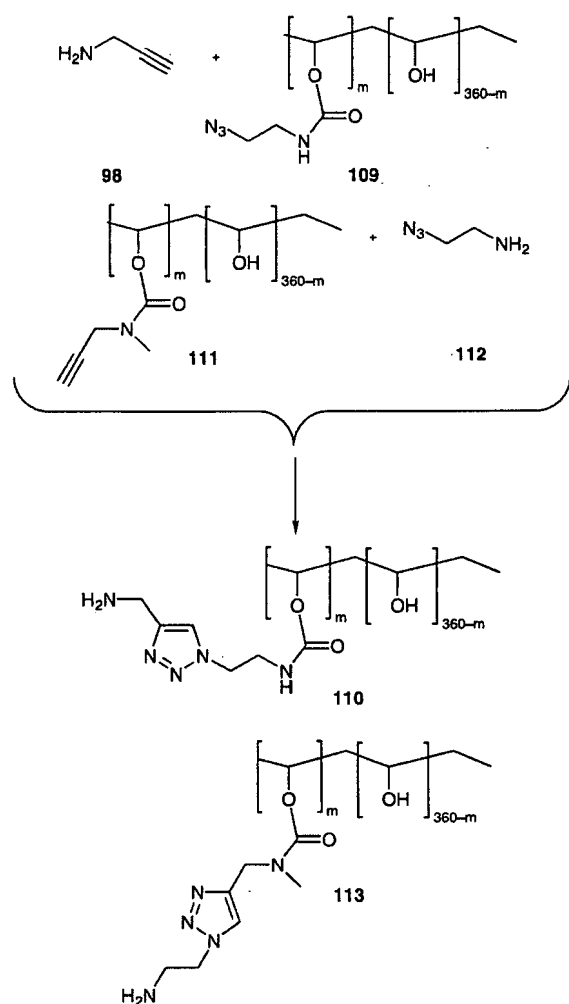
Scheme 30

Synthetic glycopolymers are currently the subject of increasing attention;[112,113] the reason is that, while simple monosaccharides can only establish weak interactions with protein receptors and elicit only a weak response to in vivo events, these interactions can be enhanced if car-

bohydrates are used in the form of macromolecular glycoconjugates. In fact, natural carbohydrate–protein interactions take place through higher-order oligomeric structures, and this observation is known as the 'cluster glycoside effect'.[114]

Du's group reported the convergent synthesis of C_3-symmetric (1→6)-N-phthalimido-β-D-glucose-derived octadecasaccharide **116** in a 62% yield (Scheme 31);[115] the title compound was obtained by the coupling of azide **114**, comprised of a hexasaccharide and an aliphatic spacer, with alkyne **115** in the presence of copper(II) sulfate (2–5 mol%) and sodium ascorbate (5–10 mol%) in aqueous tetrahydrofuran at 50–60 °C. The final octadecasaccharide **116** showed good antitumor activity in preliminary mouse tests.[115]
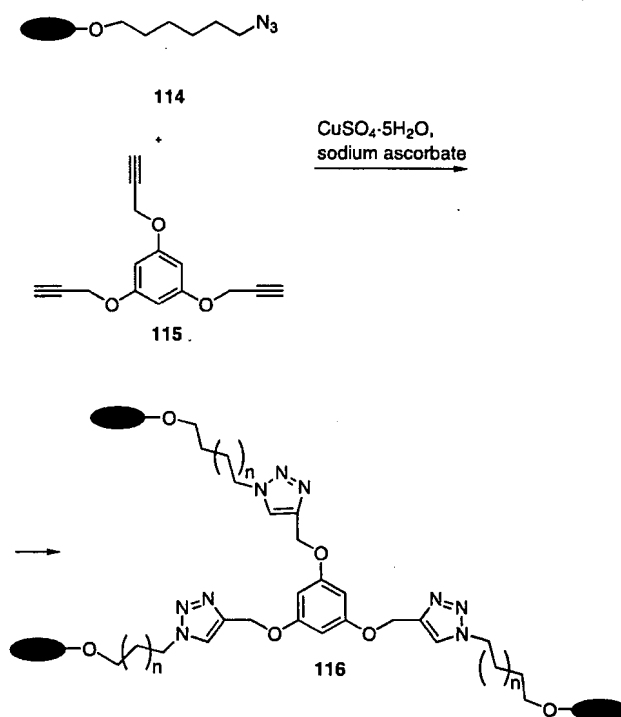
Scheme 31

Triazole rings were also made in the direct assembly of functionalized sugars by reaction of alkynyl and azido glycosides. The assembly proceeded to completion in reaction times no longer than 45 minutes when organic-soluble copper(I) complexes such as tris(triphenylphosphine)copper(I) bromide [(Ph$_3$P)$_3$·CuBr] and triethylphosphite–copper(I) iodide complex [(EtO)$_3$P·CuI] were used under microwave irradiation.[51]

The functionalization of polyalkyne materials by co-clicking reactions of appropriate mixtures of mannose- and galactose-based azides has been reported[116] as a simple and efficient route to synthetic libraries of materials that differ only in the nature of the sugar moiety. In this procedure, (PPh$_3$)$_3$CuBr and N,N-diisopropylethylamine were used as the catalytic system. ^{1}H NMR and FT-IR analysis confirmed that the conversion of the alkyne groups of **117**

into triazoles was achieved in nearly quantitative yield, and the molecular weight distribution of glycopolymers **120** was not significantly variable (Scheme 32).[116]

Scheme 32

123 R = α-mannopyranoside
124 R = β-galactopyranoside
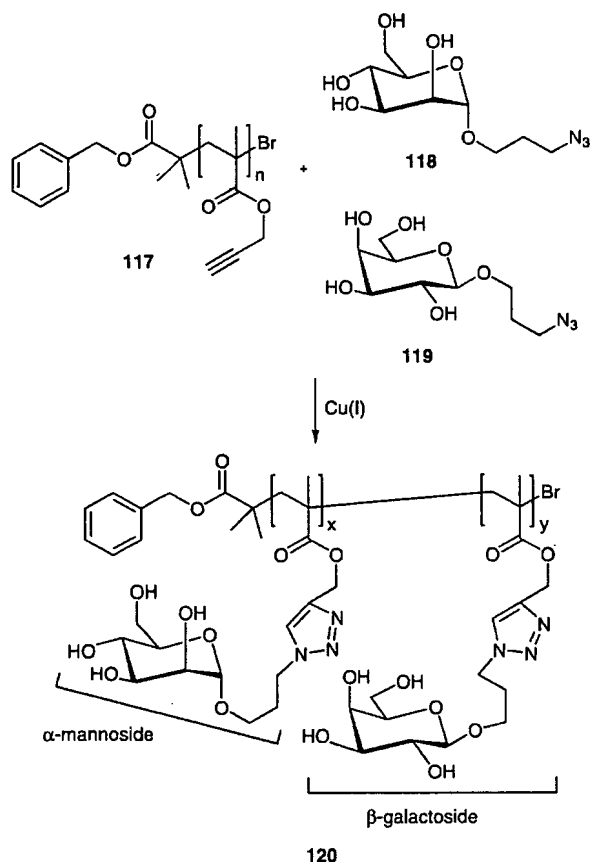
Scheme 33

A visibly fluorescent tag was also *co-clicked* into a polymeric scaffold via fluorescent azide **121** (Scheme 33).[116] The resulting polyvalent ligands **123** and **124** are quite useful in protein–carbohydrate binding interaction studies.[117] The reported glycopolymers were subjected to binding studies with model lectins, that is, a series of plant proteins that selectively binds to carbohydrates; in particular, concanavalin A and *Ricinus communis* agglutinin bind to mannose and galactose, respectively.[116]

6-Azido-6-deoxycellulose (**125**), obtained by low-temperature tosylation of parent cellulose and subsequent nucleophilic displacement by sodium azide in *N*,*N*-dimethylformamide, was subjected to copper(I)-catalyzed Huisgen cycloaddition with a series of acetylene derivatives. Thus, cellulose analogues **126–128**, bearing methoxycarbonyl, 2-aniline, and 3-thiophene motifs (Figure 2), were prepared in a selective and efficient manner.[118]

Tetrazole-containing polymers are also of interest for the production of ion-exchange and chelating resins and superabsorbents.[119] This novel class of polymers can be obtained by means of click chemistry as a post-polymerization modification of polyacrylonitrile derivatives.[119] The starting polymeric materials can be poly(ho-

moacrylonitrile), random styrene–acrylonitrile (SAN) copolymer, block copolymer of acrylonitrile and styrene, and also silica or polystyrene particles grafted with poly-SAN. These polymeric materials are prepared by atom-transfer radical polymerization (ATRP),[120] a technique for controlled radical polymerization. This technique yields halogen-terminated polymers that can be further used as
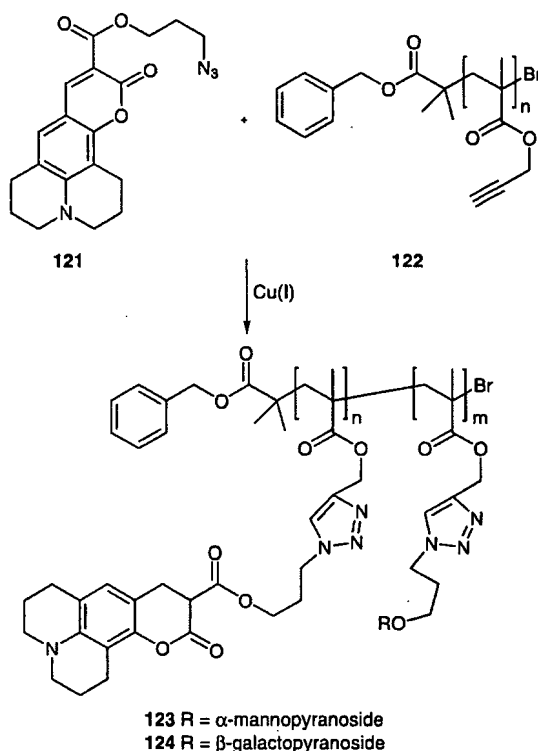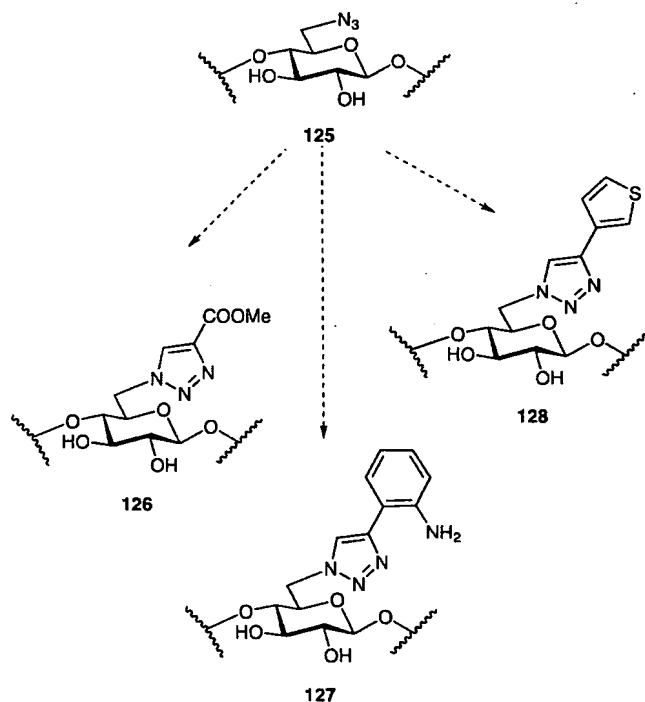
Figure 2

macroinitiators for the synthesis of block copolymers or as precursors of other chain-end-functionalized polymeric materials.

4.2 Liquid Crystals

The first example of a liquid crystal that incorporated a regioisomeric 4-substituted 1,2,3-triazole in its structure was recently reported.[16] Target compounds were designed to present cholesteric and ferroelectric mesophases through the incorporation of units in the mesogenic core that had the potential to confer such properties. In order to obtain the desired 1,4-disubstituted triazole, azide **129** (or **134**) was added to a suspension of alkyne **130** (or **135**), copper(I) iodide, and triethylamine in refluxing aqueous ethanol (Scheme 34). After 48 hours, the corresponding chiral liquid crystal was isolated in moderate to good yield (60–90%).

Thermal properties of the final compounds were investigated by using polarizing optical microscopy (POM) and differential scanning calorimetry (DSC). [16] The analysis of the mesomorphic behavior showed that by displacing the 1,2,3-triazole ring to a central core position, the melting point is substantially lowered, thereby favoring the smectic phases.

4.3 Self-Assembled Monolayers

Self assembled monolayers (SAMs) are of significant interest in interfacial reactions and many technological applications such as sensors, catalysis, microarrays, and molecular electronics.[121–123] A wider use of SAMs in tech-

nology requires, as a first important step, the search for versatile organic reactions that can be performed on them, allowing for the introduction of required functionalities to the surface. For this purpose, click chemistry seems to be a good option.

The 1,3-dipolar cycloaddition of azides and alkynes to form 1,2,3-triazoles has been used to couple monolayer-covered azide-terminated silica substrates with acetylenes.[124] The development of this reaction would permit the attachment of desired functionalities needed for specific surface properties in these alkylsiloxane monolayers. Azide-terminated monolayers were prepared in situ from 11-bromoundecylsiloxane monolayers and were subsequently reacted with three different acetylene compounds, as depicted in Scheme 35.[124]

The reactivity of acetylenyl-terminated SAMs on gold towards click chemistry has also been investigated.[125,126] To a reaction mixture containing the acetylenyl-terminated SAM-coated gold substrates in aqueous ethanol were added an azido compound, copper(II) sulfate, and sodium ascorbate, and the mixture was stirred at room temperature for 12 hours (Scheme 36). This procedure proved to be a mild and efficient way of tailoring surface functionalities.[125]

In a similar manner, the azido groups existing in mixed assembled monolayers on gold electrodes from various ω-functionalized alkane thiols and azido alkane thiols, in the presence of copper(I) catalysts, reacted rapidly and quantitatively with terminal acetylenes to form 1,2,3-triazoles via click chemistry (Scheme 37).[127] The coupling reaction proceeded in high yield and under very mild conditions.

Scheme 34

Scheme 35

R¹–C≡C–R²

$R^1 = C_6H_{13}$, $R^2 = H$
$R^1 = COOMe$, $R^2 = H$
$R^1 = R^2 = COOEt$

SiO_2

SiO_2

Scheme 36

RN_3
$CuSO_4 \cdot 5H_2O$
sodium ascorbate

H_2O–EtOH

Au

Au

R =

—COOH

—O—O—O—NH₂

Me

HO

Scheme 37

$Br(CH_2)_{11}SH$

NaN_3

R =

Fe

NO_2

Me

NH
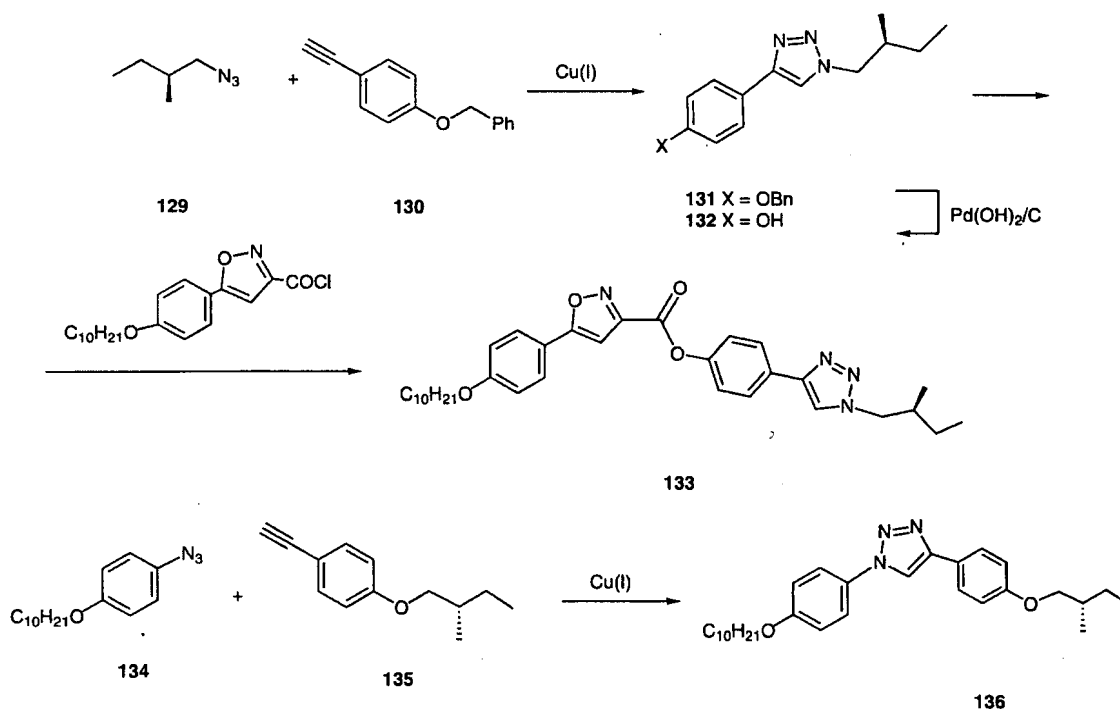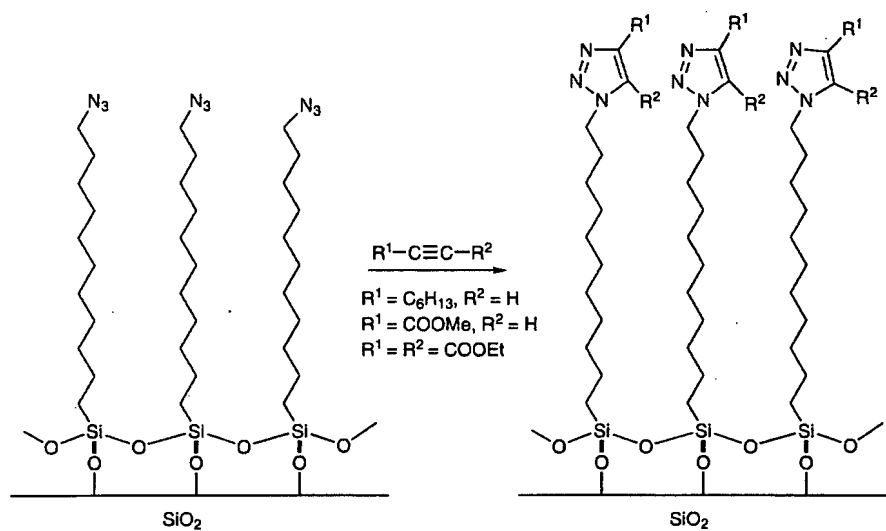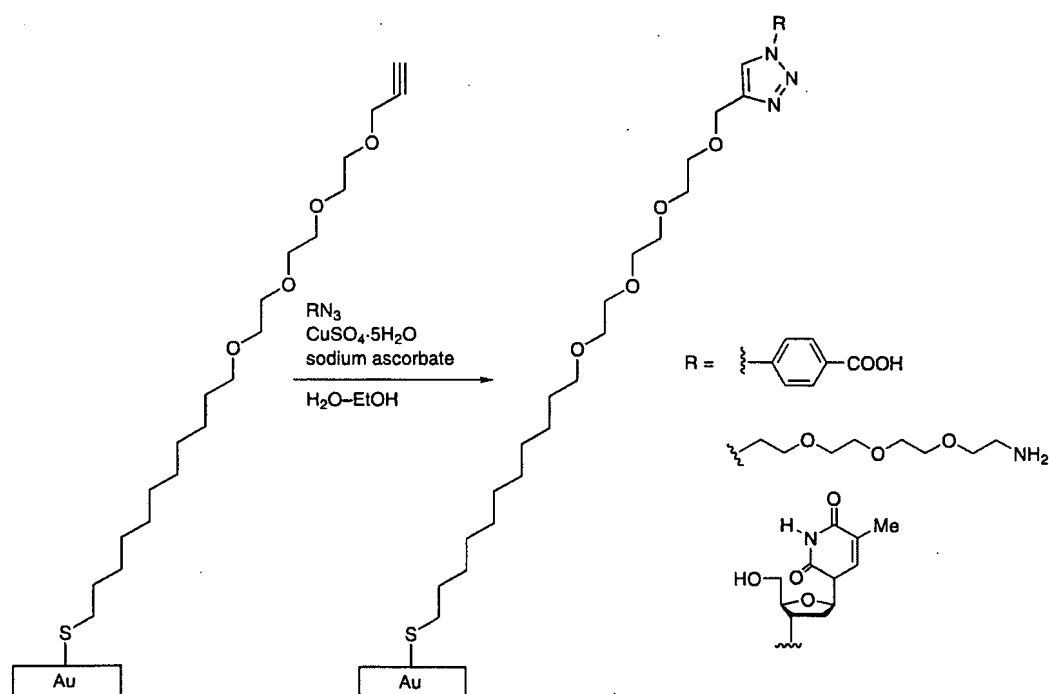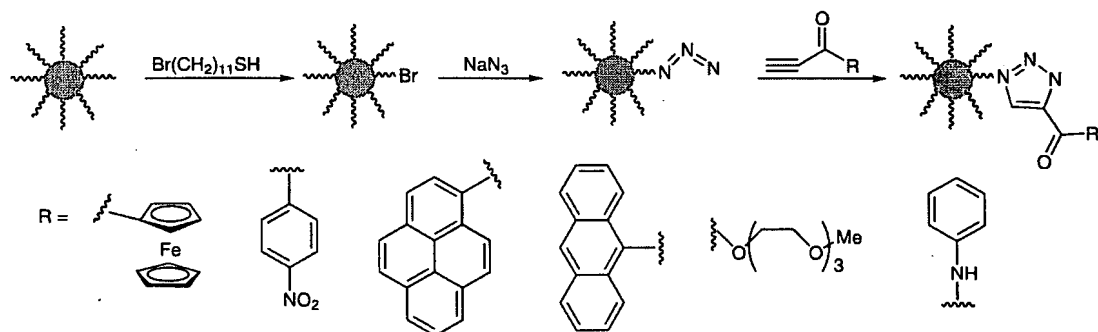
Surfaces containing organic azides are excellent platforms for further modification. It is possible to easily control and measure the amount of a surface-bound azide that, once incorporated, reacts quantitatively with acetylene. This action is carried out by monitoring the intense infrared stretching mode of organic azides. The major limitation in these reactions appears to be steric hindrance.

4.4 Multicomponent Reactions

The increasing demand for novel biologically active small molecules and the laborious process of lead discovery have made the synthetic efficiency of multicomponent reactions very attractive to organic chemists. [128,129] In these reactions where more than two starting materials react to form a product which incorporates essentially all of the reagent atoms, the selectivity of procedures and the compatibility between different functional groups represent key aspects to be considered. [130] Click chemistry, and more specifically, the Huisgen 1,3-dipolar cycloaddition, has emerged as a simple, fast, and efficient approach to synthesize functionalized 1,2,3-triazoles. For obvious reasons, the combination of both these techniques could be an important strategy for the generation of novel compound libraries. [131,132]

One of the first contributions to the multicomponent variant of the copper-catalyzed Huisgen cycloaddition was reported by van der Eycken. [133] In this process, azides were generated in situ from the corresponding halides, and underwent cyclization with copper(I) acetylides (t-BuOH–H_2O, 1:1), furnishing the expected 1,4-disubstituted 1,2,3-triazoles (Scheme 38). Performing both steps under microwave irradiation (100 W and 125 °C) significantly reduced the reaction time. The copper(I) catalyst was prepared by the disproportionation of the copper(0) and copper(II) species. The final products were obtained in roughly 90% yield.

R^1 = aryl, H   R^2 = Ph
X = Cl, Br, I
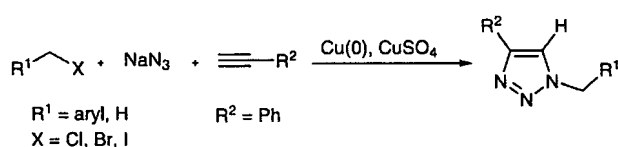
Scheme 38

Barbas and Ramachary accomplished the preparation of a proline/copper(I)-catalyzed spirotrione-triazole **148** in a 90% yield. [131] This synthesis is based on the stereospecific assembly of simple substrates like phosphorane, aldehydes, cyclic 1,3-diketones, and azides under organo-copper(I) catalysis by means of Wittig/Knoevenagel/Diels–Alder/Huisgen cycloaddition reaction sequences in a one-pot fashion (Scheme 39).

The Huisgen cycloaddition has also been combined with the Biginelli multicomponent reaction [134] for the efficient synthesis of libraries of compounds such as **149**. [132] This was carried out by performing rapid and high-yielding microwave-assisted azide–acetylene couplings. For this purpose, readily available 6-(azidomethyl)dihydropyrimidones **150** were prepared as key intermediates using a multicomponent approach, and then reacted with a series of terminal acetylenes under copper(I) catalysis. The best results were obtained when microwave irradiation was used, heating at 80–100 °C. This led to an attractive linkage of two important N-heterocyclic pharmacophores (Scheme 40) with four points of diversity that can easily be accessed by choice of the appropriate building blocks. [132]

4.5 Creating Drug Candidates under *Click* Conditions

The traditional process for the design and discovery of a new pharmaceutical drug is both time-consuming and expensive, despite the development of combinatorial chemistry. [1] Thus, the revolutionary click chemistry concept is being exploited in drug and biomedical research, [17,135–137] as click chemistry constitutes a powerful tool for the rapid discovery of target enzyme inhibitors that exhibit great potency and specificity. [138,139] In this context, Lee and co-workers prepared a library of triazole compounds that were shown to be potent inhibitors of human α-1,3-fucosyltransferase VI, a pivotal enzyme which is involved in the catalysis of the final glycosylation step in the biosynthesis and expression of many important saccharides. [140] Srinivasan and co-workers accomplished the preparation of a vast series of enzymatic inhibitors in processes related to obesity and diabetes. [141]

Furthermore, *click* adducts have been employed, not only for the development of new drug candidates, but also in glycobiological studies, such as DNA sequencing [142] or the coupling of ligands to vesicles and liposomes. [143]

The triazole scaffolds are expected to be more than just passive linkers, as they might be associated with biological targets through intermolecular hydrogen-bonding and dipole interactions. [17]

It is reasonable to postulate that the combination of heterocyclic pyrazinones and triazoles could lead to compounds with pharmacological interest, as both structural motifs have been reported to exhibit biological activities. [144,145] In this context, Kaval et al. considered two different synthetic approaches, [146] the first of which was 1,3-dipolar cycloadditions with acetylene-containing 2(1H)-pyrazinones **153**. In this case, the active catalyst was generated in situ from copper wire and copper(II) sulfate, and the reaction was carried out with microwave irradiation in aqueous *tert*-butyl alcohol (Scheme 41).

The other approach was a 1,3-dipolar cycloaddition with azide-containing 2(1H)-pyrazinones, which are in equilibrium with the corresponding tetrazoles via an intramolecular cyclization that involves the vicinal pyrimidinic nitrogen (Scheme 42). [146] This equilibrium was found to be shifted to the bicyclic derivative, thus it was assumed that an increase in temperature should reverse the equilibrium to the unstable azide. Nevertheless, microwave-

Scheme 39

R[1] = H, Me
R[2] = Me, aryl, heteroaryl
R[3] = Et, *i*-Pr, Bn
R[4] = aryl, heteroaryl

Scheme 40

mediated reaction attempts resulted either in no reaction or in reagent decomposition.

Surprisingly, when the reaction was carried out at room temperature using a tertiary amine as a copper(I) stabilizing agent, the desired substituted triazoles were obtained in moderate to good yields (Scheme 42).[146]

R[1] = CH₂C₆H₄OMe
R[2] = H
R[3] = 4-MeO, 4-NO₂, 4-NMe₂, 4-Cl, 3,5-(CO₂Me)₂, 4-CONH₂

Scheme 41

The Huisgen [3+2] cycloaddition has also been employed in the search for inhibitors of acetylcholinesterase (AChE), a pivotal central nervous system neurotransmitter.[147,148] For this purpose, a special technique, named target-guided synthesis (TGS)[149] has been used; this approach is based on the assembly of two building blocks, with weak affinity for different structural features of the target enzyme, to produce a bivalent inhibitor.

Thus, in situ click chemistry between alkynes and azides with chains of differing length was used, without catalyst but instead in the presence of the enzyme as a template. Under these conditions, only those building blocks which are involved in an interaction with the active site of the en-

R^1 = Ph, 4-MeOC$_6$H$_4$
R^2 = H, 4-MeOC$_6$H$_4$, 4-MeO$_2$CC$_6$H$_4$
R^3 = Ph, CO$_2$Et, CH$_2$NMe$_2$, CH$_2$OH, CH$_2$CH$_2$OH, C(Me)$_2$OH, C(Me)(Et)OH
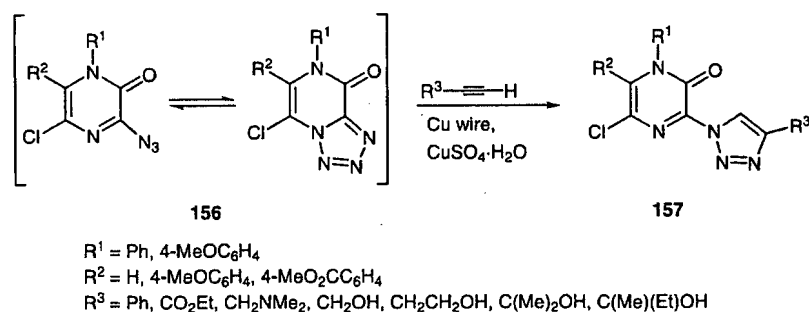
Scheme 42

zyme are close enough to each other to react and produce the potent inhibitor. It is remarkable that only *syn*-derivatives, that is, 1,5-disubstituted triazoles, were formed from among all the possible combinations in the presence of the enzyme; this is in contrast to what happens under thermal Huisgen conditions, wherein, as indicated above, a roughly 1:1 mixture of regioisomers is obtained.[48]

Some of these compounds exhibit femtomolar inhibition of AChE, the lowest dissociation constants reported to date for non-covalent inhibition of this enzyme.[147,148] Triazole derivatives such as **158** (Figure 3) simultaneously mimic the structure of three AChE inhibitors: propidium and decamethronium ions, and tacrine.[150,151]

158
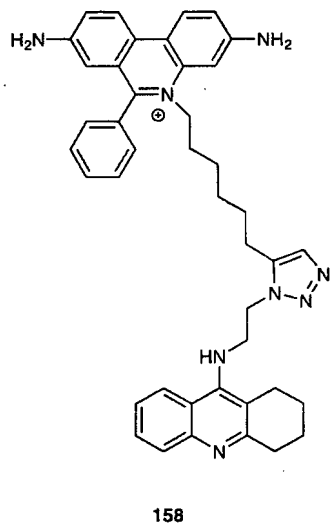
Figure 3

The in situ click chemistry approach has also been successfully applied to the formation of HIV-1 protease inhibitors; for this purpose, alkyne **159** and azide **160** were incubated in the presence of the protease, HIV-1-PR (Scheme 43). This protease, which plays an important role in the inhibition of viral replication, itself acts as a template for the reaction and even increases the rate of formation of the *anti* 1,4-triazole product **161**.[152]

Other examples of an enzyme-mediated preparation of triazole derivatives were reported by the groups of Sharpless[153] and Gmeiner[154] for the preparation of carbon-

159   **160**

HIV-1-Pr

161
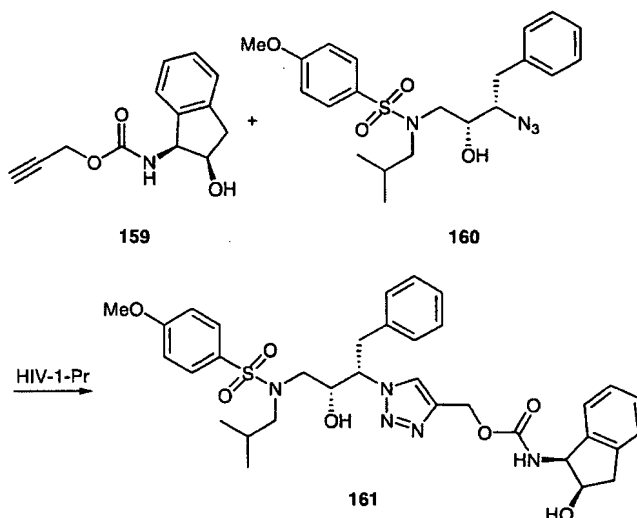
Scheme 43

ic anhydrase II inhibitors and dopaminergic agonists, respectively.

Another way of fighting against AIDS infection is the blockage of a viral envelope protein to antigens in the host T-cell surface.[155] Gopi et al. carried out the modification of a proline unit with 4-phenyl-1,4-disubstituted 1,2,3-triazole, formed through a *click* [3+2] cycloaddition reaction.[156] This led to a peptide (Figure 4) that binds to the viral protein with an affinity two orders of magnitude greater than that of the parent peptide and, thus, strongly disrupts the interaction of this glycoprotein with the host cell surface.[156]

native peptide with proline   peptide with substituted proline

Figure 4
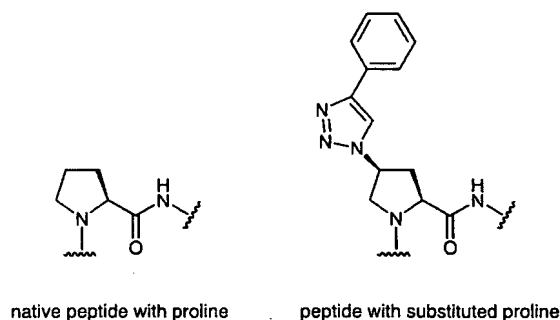
tag = rhodamine—biotin
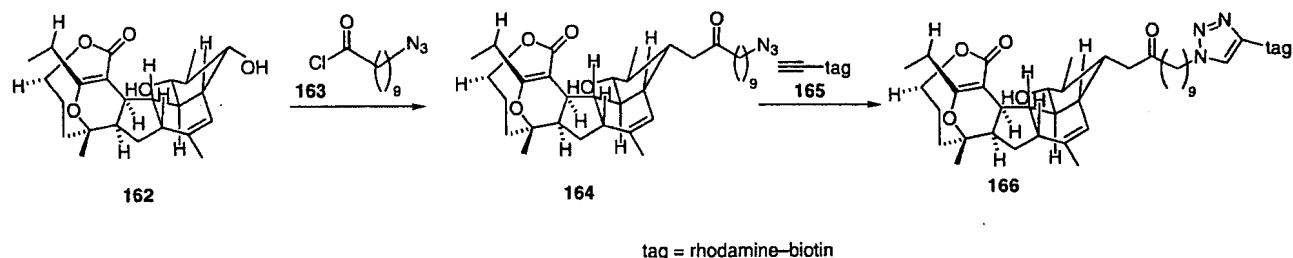
Scheme 44

In the search for inhibitors of carboxylesterase I (CE-I), an enzyme involved in some important biological processes such as drug metabolism, a series of rhodamine–biotin-tagged forms of (–)- and (+)-**166** were prepared (Scheme 44). These compounds were obtained by the copper(I)-catalyzed reaction of **164** with an alkyne-derivatized rhodamine–biotin agent **165**. Binding studies revealed that association of (–)-**166** with CE-I was stable even to protein denaturation.[157]

Click chemistry has also been applied toward the synthesis of resveratrol (**167**), a polyphenolic compound found in some plants, such as in the skin of red grapes.[158] This compound exhibits some beneficial properties, including cardioprotective, neuroprotective, antiviral, and anti-inflammatory properties,[159] so derivatives of resveratrol are of interest as potential biologically active compounds. Pagliai et al. accomplished the preparation of a library of resveratrol derivatives **168–172**, wherein the carbon–carbon double bond of the stilbene moiety was replaced with a triazole ring (Figure 5).[160] After the evaluation of the cytotoxicity of these compounds, the preliminary biological analysis suggested that some of the compounds screened were more potent cytotoxic and antiproliferative agents than resveratrol itself.

The broad spectrum of biological activities exhibited by vitamin D[161] has prompted researchers to carry out the

synthesis of analogues such as the triazole-bearing vitamin D.[162] Click chemistry allowed for the cycloaddition of a vitamin D side-chain terminal acetylene with phenyl azide, and separately with a vitamin D side-chain azide, to yield the corresponding 1,2,3-triazole analogues of 1-α-hydroxy-vitamin D_3 and a dimeric vitamin D derivative (**173**), in which the monomeric units are linked through a triazole tether (Figure 6).[163]

173

Figure 6

Scheme 45

resveratrol (**167**)
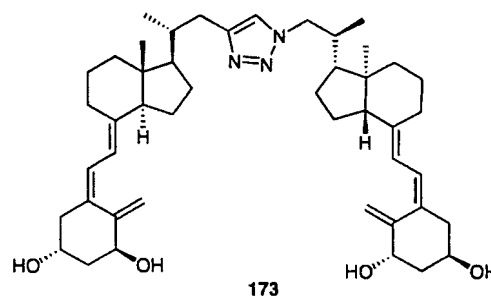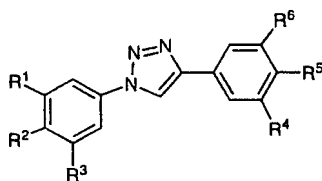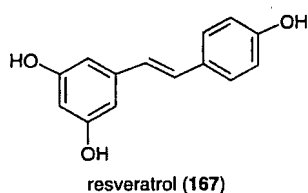
168, R^1 = OH; R^2 = H; R^3 = OH; R^4 = H; R^5 = OH; R^6 = H
169, R^1 = H; R^2 = OH; R^3 = H; R^4 = OH; R^5 = H; R^6 = OH
170, R^1 = OMe; R^2 = H; R^3 = OMe; R^4 = H; R^5 = OMe; R^6 = H
171, R^1 = H; R^2 = OMe; R^3 = H; R^4 = OMe; R^5 = H; R^6 = OMe
172, R^1 = OH; R^2 = H; R^3 = OH; R^4 = H; R^5 = OMe; R^6 = H
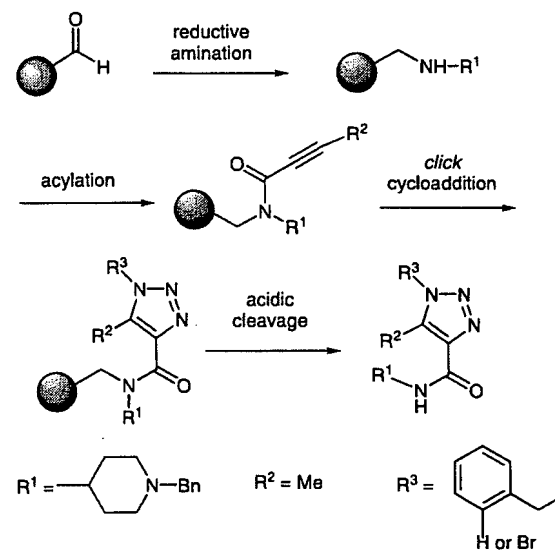
Figure 5

Taking advantage of the *click* strategy, several functionalized resins were prepared and evaluated for the parallel solid-phase synthesis of a series of aryl carboxamides, which frequently serve as key pharmacophoric elements in drugs.[164] In this context, Gmeiner and co-workers reported the preparation of a library of solid-supported *N*-benzyl 1,2,3-triazole carboxamides.[165] These compounds

174
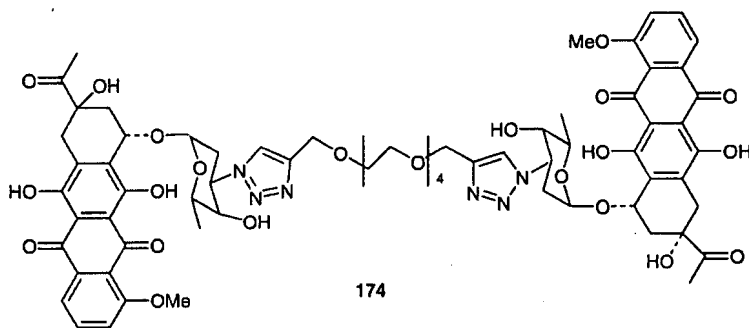
Figure 7

were prepared as depicted in Scheme 45, in four steps: reductive amination of a benzaldehyde derivative, *N*-acylation with alkynoic acids, [3+2] cycloaddition, and a final acidic cleavage. All target compounds thus obtained were screened for G-protein coupled-receptor binding, and some demonstrated excellent receptor recognition.[165]

Another target for *click* reactions is daunorubicin, one of the most important drugs employed in cancer chemotherapy, whose possible mechanisms of action include, among others, DNA intercalation.[166] In order to increase the drug's DNA binding affinity, dimeric species derived from daunorubicin derivatives offered the possibility of preparing a novel series of anticancer analogues.[137] Thus, through *click* reactions, several dimeric derivatives such as **174** (Figure 7), bearing triazole tethers with differing chain lengths and varying flexibility, were successfully synthesized using (EtO)₃PCuI as catalyst. Cytotoxicity studies revealed that shorter linkers presented a stronger activity against cancer cells, with flexibility being another key aspect.

Bitriazolyl compounds **177**, which are of interest because of potential use in agrochemical research as well as in materials science, were synthesized through a copper(I)-catalyzed Huisgen cycloaddition by using the azidotriazole

175 and several terminal acetylenes as starting materials, followed by aminolysis of the ester moiety (Scheme 46). Tobacco mosaic virus was used as a model system for testing the antiviral activity of these bitriazolyl products, and some were shown to be even more potent than commercial products used in the treatment of this agricultural pest.[167]

175   R = alkyl, aryl, carboxyl   **176**

177
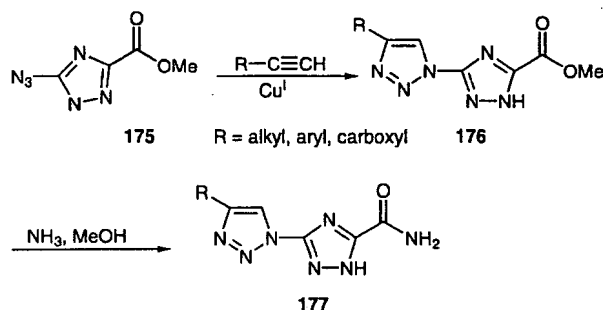
Scheme 46

Another interesting target for the design of new pharmacological drugs is the modulation of protein–carbohydrate interactions; for example, inhibitors of galectins (β-galactoside-binding lectins), might be potential active agents in

photoaffinity probe
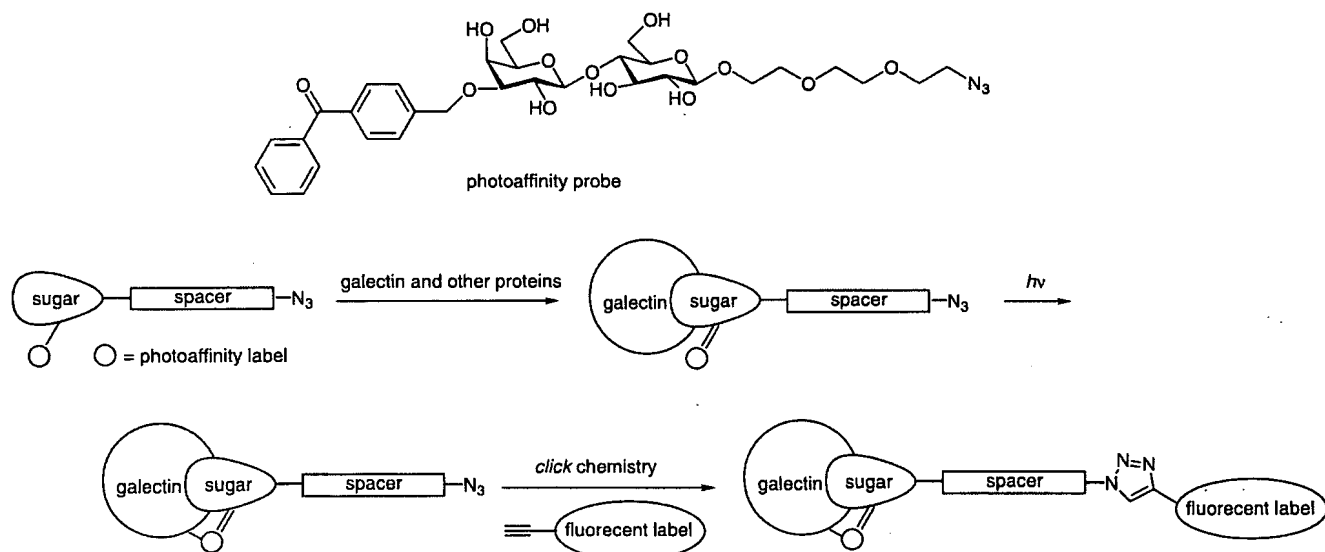
Scheme 47

cancer treatment.[168] Pieters developed a method for the detection of galectins, even mixed with other proteins (Scheme 47).[169] For this purpose, he used a benzophenone-based photoaffinity label, in which the benzophenone group was bound to the C-3 position of the galactose residue of lactose, and the glucose moiety of lactose was derivatized with a polyether chain that had a terminal azido group. When binding of galectin and the modified disaccharide took place, irradiation of the system resulted in an interaction of the photoaffinity label with the protein, followed by triazole formation, thus enabling galectin detection.[169]

Another example of protein–carbohydrate interaction studied with triazole derivatives was reported by van der Peet et al.[170] The authors proved that only α-configured mannose derivatives bearing triazole substituents could act as substrates for Leishmania β-1,2-mannosyltransferases.

4.6   Click Chemistry with Peptides and Proteins

Methods are needed for profiling the activity of enzymes in vivo in order to understand the role that these proteins play in physiological and pathological processes. Activity-based protein profiling (ABPP)[171] uses active-site-directed chemical probes to determine the functional state of enzymes in complex proteomes, distinguishing active enzymes from their inactive precursors and/or inhibitor-bound form. Scheme 48 illustrates a standard ABPP probe, consisting of a reactive group and one or more reporter tags, the size of the latter being the main limitation

for the in vivo application of ABPP probes.[172] In contrast, click chemistry ABPP allows for the profiling of living cells and organisms by treating them with tag-free azide- or alkyne-modified probes, which are then conjugated in vitro to the complementary alkyne- or azide-modified tag under cycloaddition reaction conditions to visualize probe-labelled proteins (Scheme 48).

Cycloaddition-based ABPP is a versatile method that allows, for example, for the in vivo and in vitro labelling of enzymes and for their detection from among the whole proteome; this methodology gives access to in vivo studies to determine whether a certain drug inhibits its target.[173] The combination of both approaches, ABPP and click chemistry, has contributed to the design and synthesis of potent and selective fatty acid amide hydrolase inhibitors.[174]

A further approach to aid in the study of protein binding and function is the incorporation of synthetically modified amino acids or peptides into the parent structure.[175,176] In this sense, model studies have also been performed for the C-terminal lipidation of proteins by exploiting the Huisgen cycloaddition.[177]

For instance, Marik and Sutcliffe reported the [18F]-labelling of peptides via click conjugation of ω-[18F]-fluoroalkynes to several peptides that bear azide moieties, to furnish the corresponding [18F]-labelled target peptides (Scheme 49).[178] Derivatives such as 180 are widely used as in vivo imaging agents of various physiological and pathological processes, using positron emission tomography.[178]

standard ABPP
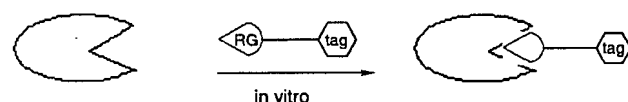
click chemistry ABPP
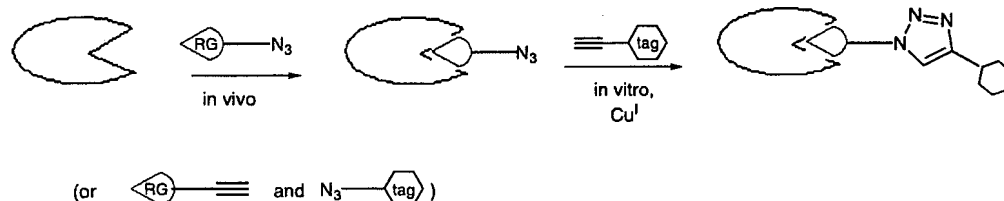
RG = reactive group

Scheme 48

178   **179**   **180**
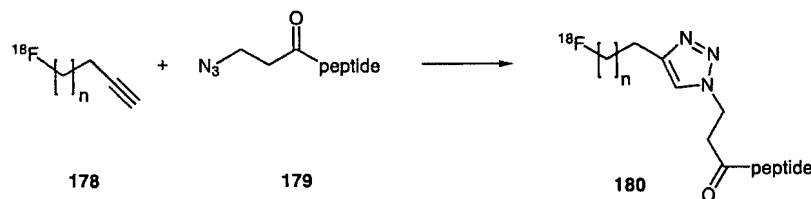
Scheme 49
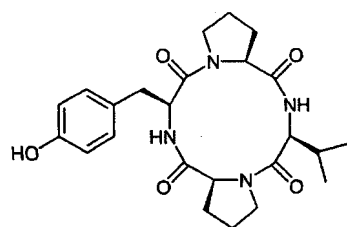
tyrosinase inhibitor, **181**
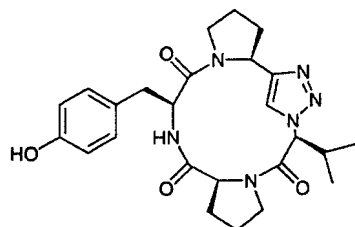
triazole analogue, **182**

Figure 8

An additional example that demonstrates the enormous potential of click chemistry connected with proteins is the preparation of small cyclic peptide analogues that are too strained for ring closure via lactamization. In order to demonstrate such a cyclization of peptides, the triazole analogue **182** of tyrosinase inhibitor **181** (Figure 8) was synthesized.[179]

Thus, compound **182** was prepared starting from compounds **185** and **186** (Scheme 50), which were deprotected and coupled to afford the linear tetrapeptide analogue **184** that was then subjected to *click* conditions and thereby yielded **182**.

Carbohydrate-derived azides have also been used in the structural modification of peptides (Scheme 51).[180] Thus, coupling of per-O-acetylated glycopyranosyl azides **187** with bromoalkynamide **188** gave the corresponding glycopyranosyl triazole derivative **189**.

188

187

Cu[i]

189

R = Ac, (OAc)₃-β-GlcNAc-

R = Ac, $(OAc)_3$-β-GlcNAc-

Scheme 51

b: triazole formation

a: peptide bond formation

Triazole analogue, **182**

a   *b*

183

184

185

186

Scheme 50

190

191
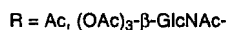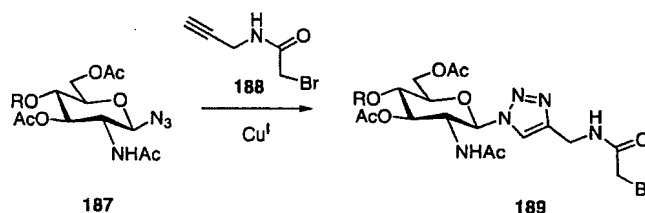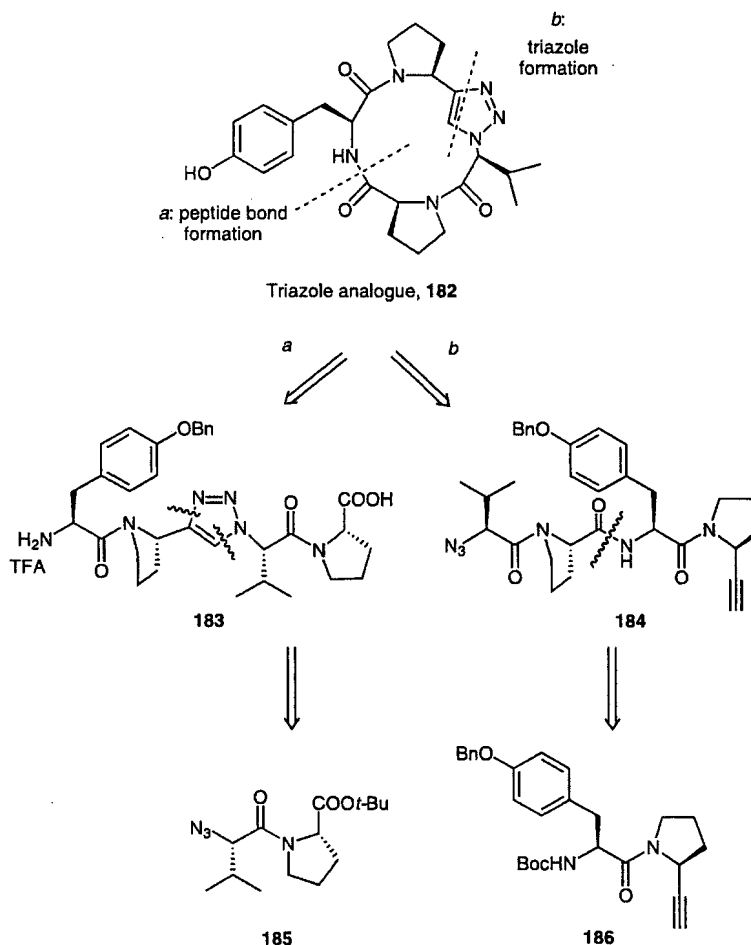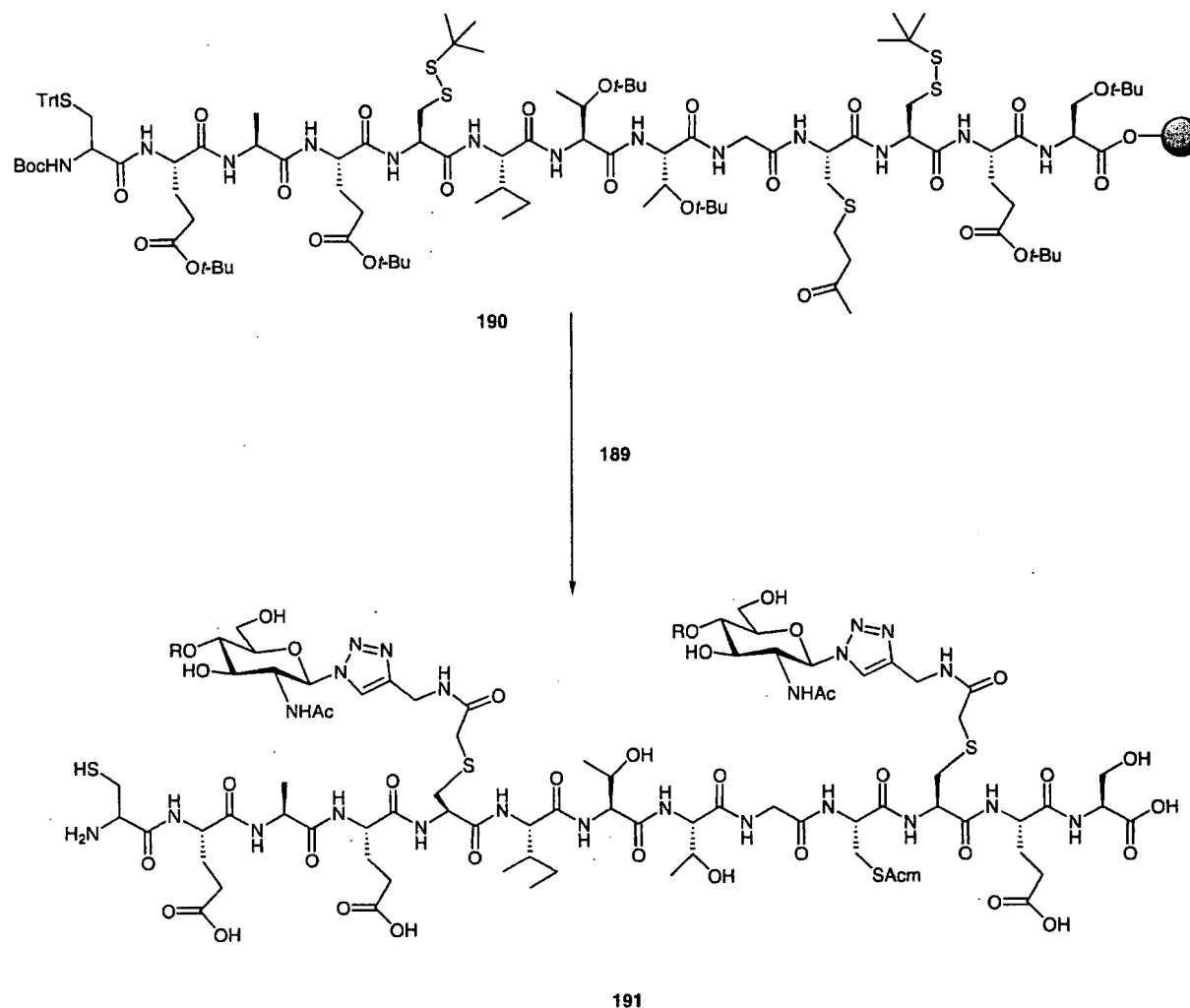
Scheme 52

The bromine atom in **189** was displaced through nucleophilic attack by the thiol groups of the cysteine units in peptide **190** (Scheme 52).[180] Subsequent deprotection steps yielded the target glycotriazole-functionalized peptide **191**.

Biomolecules can be subjected to immobilization in their native state by the use of some *click* reactions like Diels–Alder and [3+2] cycloadditions. In this context, Sun and co-workers reported the use of a bifunctional poly(ethylene glycol) linker with an alkyne terminal group (Scheme 53)[181] for the immobilization of both proteins and carbohydrates.

4.7   DNA as Participant in *Click* Reactions

Click chemistry has successfully been evaluated as an easy and efficient method for DNA alkylation.[182] The interest in this chemical modification is because of the importance of biological methylation in regulatory mechanisms of gene transcription. Thus, the coupling of the nucleoside analogue **192**, which bears an alkyne moiety, with an organic azide, accompanied by covalent bond formation with a DNA fragment mediated by a methyltransferase enzyme, proceeded in an efficient manner and

glass slide
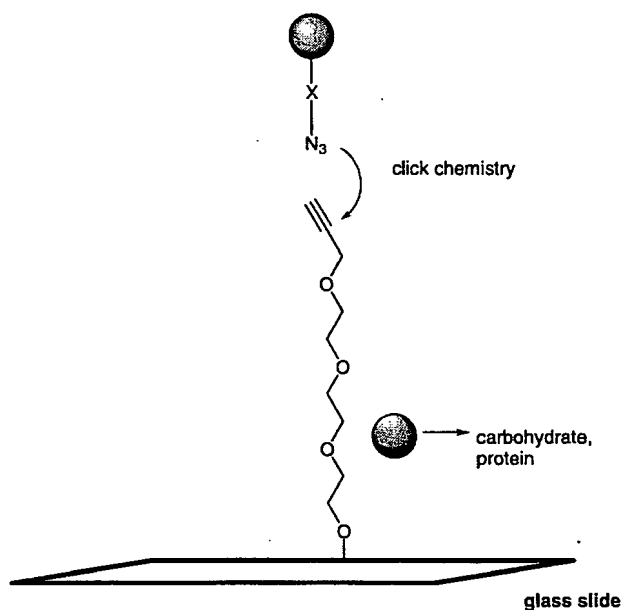
Scheme 53

effectively demonstrated the possibility of using DNA in *click* modifications (Scheme 54).[182]

192 → **193**
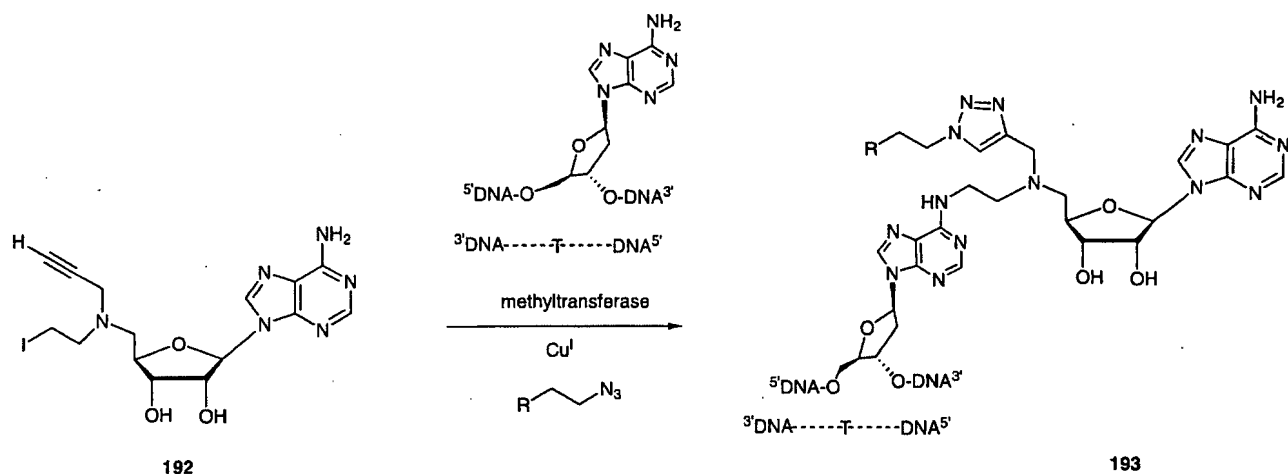
Scheme 54

The modification of either a 3'- or 5'-terminus or an internal position of an oligonucleotide with a primary alkylamine group is a widely used method for the introduction of additional functional groups into DNA.[183] In this sense, the oligonucleotide 5'-amino-GTT TTC CCA GTC ACG ACG-3' was used to prepare the azido-labeled DNA **194**, which by coupling with the alkynyl 6-carboxyfluorescein

195 led to a fluorescent oligonucleotide with high selectivity, yield and stability (Scheme 55).[184] This kind of derivative can be detected with laser-induced fluorescence techniques.[184]

In the search for DNA metallation procedures, in order to increase the conductivity of these modified DNA nanostructures, an efficient and selective method for the depo-
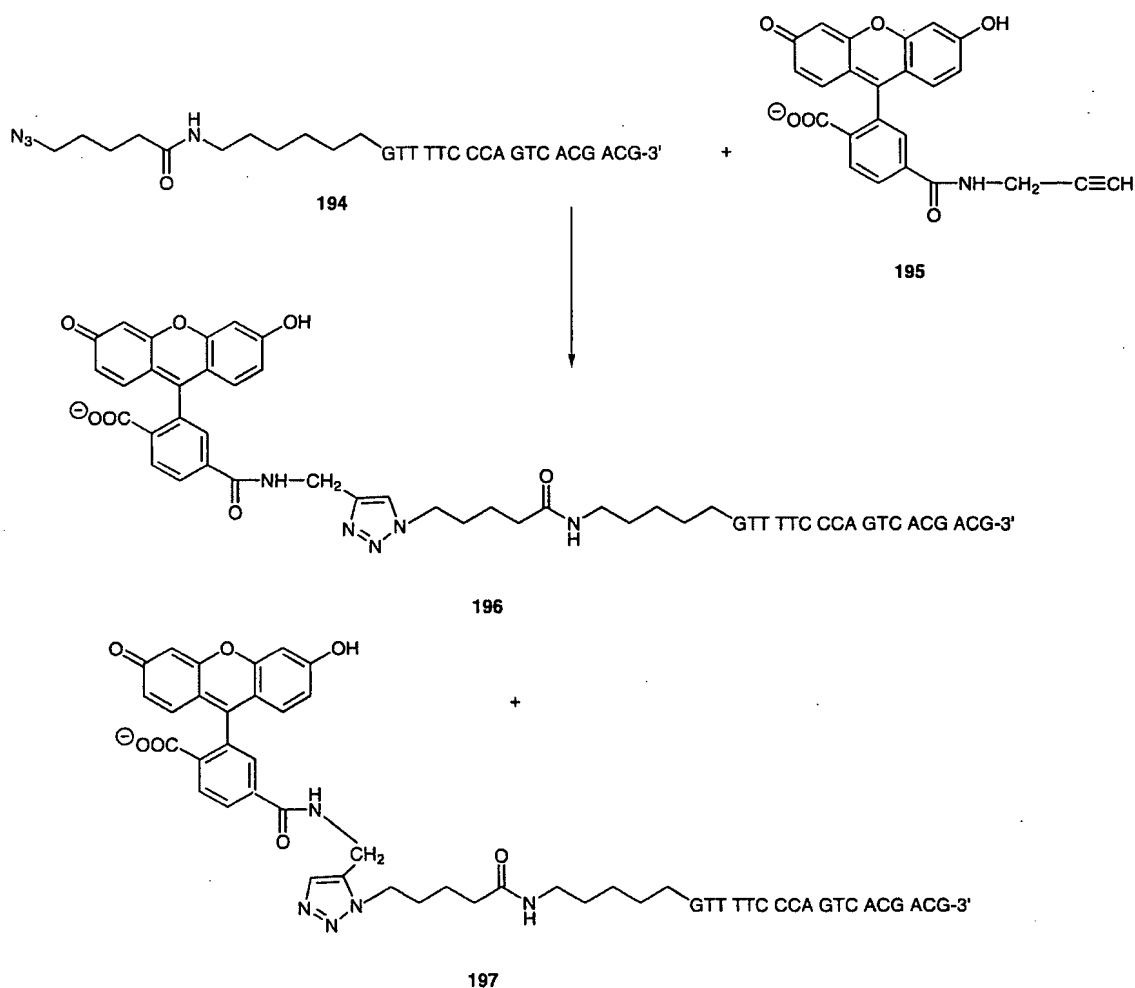
Scheme 55

sition of silver(0) around aldehyde-containing DNA has been achieved.[185] Acetylene-labelled nucleotide triphosphates were inserted by using DNA polymerases and the product DNA was employed in a *click* approach with aldehydo azides; the latter process can be efficiently performed on a polyacrylamide gel.[185]

5   Concluding Remarks

Click chemistry refers to a revolutionary chemical concept concerning only reliable and efficient reactions. The reason for such a term is the requirement for using valuable, high-yielding, and low-cost reactions as an optimal method for the development of substances of biological interest.

In this context, the copper-catalyzed Huisgen cycloaddition, in particular, has proven to be the best example of *click* reactions, and there is already an extensive list of reports that describe the use of this practical reaction in the preparation of glycoconjugates, potent glycosidase inhibitors, and even protein and DNA modifications. It follows, then, that the reactions described herein can help researchers to accelerate the comprehension of biological systems and to develop new active substances such as vaccines and pharmaceutical drugs. Significant advances in click chemistry are anticipated.

As additional evidence for the current vitality of this field, after completing the writing of this review, the authors became aware of two recent and insightful reviews focused on selected applications of the archetypal *click* reaction of azides and alkynes to polymer and materials science.[186,187]

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Multivalent, bifunctional dendrimers prepared by click chemistry†

Peng Wu,[a] Michael Malkoch,[b] Jasmine N. Hunt,[b] Robert Vestberg,[b] Eiton Kaltgrad,[a] M. G. Finn,*[a] Valery V. Fokin,*[a] K. Barry Sharpless*[a] and Craig J. Hawker*[b]

Unsymmetrical dendrimers, containing both mannose binding units and coumarin fluorescent units, have been prepared using click chemistry and shown to be highly efficient, dual-purpose recognition/detection agents for the inhibition of hemagglutination.

The high functional group density at the chain ends of dendrimers, coupled with unprecedented control over molecular structure, makes these synthetic materials extremely attractive candidates for a variety of surface active applications.[1] One of the most promising is to exploit the numerous chain end groups as multivalent binding sites[2] for interaction with biological receptors and cell surfaces in the construction of targeted drug delivery systems.[3] This concept has been exploited by Cloninger et al.[4] in a series of pioneering studies designed to alter and control the strength of lectin binding by varying the generation number of a series of mannose functionalized PAMAM dendrimers. For future practical applications, at least 3 functional units are required: a targeting moiety, a medicinally active agent (drug), and a diagnostic label such as a fluorescent dye, each attached at a specific position within the nanostructure. While a number of approaches have been reported for combining all of these elements into a single system,[5] the structural control and monodispersity of dendrimer-based macromolecules promise superior performance.[6] A general strategy for the facile synthesis of chemically differentiated dendrimers which allows for the introduction of functional groups at defined locations has however not been reported. To address this challenge, the synthesis of dendritic block copolymers[7] in which two distinct clusters of functionality (targeting and detection) are placed at the chain ends in a controlled fashion is described. The key chemical transformation which allows simple and facile preparation of these dual-purpose, multifunctional materials is the copper(I)-catalyzed azide–alkyne cycloaddition, a premier "click" reaction.[8] The high efficiency of this process is exploited to couple the dendritic blocks together, while its tolerance of a wide variety of functional groups allows the introduction of reactive units at the periphery without the use of protecting groups.[9]

The synthetic approach selected is based on 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) as a biocompatible building block and the resulting anhydride 1 provides easy access to both the alkyne 2 and the azide 3 by condensation with the appropriate alcohol.[10] Removal of the acetonide protecting groups and subsequent acylation with the anhydride 1 allows for facile generation growth of the dendritic blocks containing either a single acetylene 4 or azide group 5 at the focal point. The resulting dendrons can be rendered reactive and hydrophilic by deprotection or kept protected and hydrophobic by retention of the acetonide caps.

To facilitate these studies and allow preparation of a library of structures, two series of dendrons up to the 4th generation were synthesized in high yield and purity (Scheme 1). Coupling of the differentiated dendritic blocks containing a variety of chain end

[a]The Scripps Research Institute, Department of Chemistry, BCC-315
10550 N. Torrey Pines Rd., La Jolla, CA 92037, USA.
E-mail: sharpless@scripps.edu; Fax: +1 858 784 7562;
Tel: +1 858 784 7515
[b]Materials Research Laboratories, University of California, Santa
Barbara, CA 93106, USA. E-mail: hawker@mrl.ucsb.edu;
Tel: +1 805 893 7161
† Electronic supplementary information (ESI) available: Synthesis and characterization of dendritic macromolecules. See DOI: 10.1039/b512021g

Scheme 1 Synthesis of hydrophilic and hydrophobic dendrons with either acetylene 4 or azide 5 groups at the focal point.

4, FG =C≡CH , R = Acetonide

5, FG = (CH₂)₅N₃, R = H
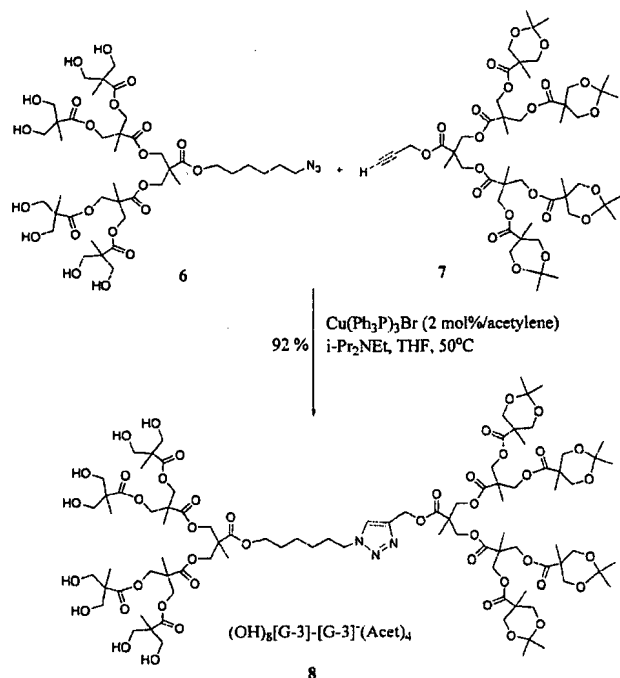
Scheme 2 Synthesis of unsymmetrical dendrimer **8** containing a chemically differentiated surface.

functional groups proceeded smoothly under the copper(I)-catalysis conditions. Thus, reaction of (HO)$_8$-[G-3]-Az, **6**, and (An)$_4$-[G-3]-Acet, **7**, in dry THF in the presence of catalytic [Cu(PPh$_3$)$_3$Br] and *N,N*-diisopropylethylamine furnished the diblock dendrimer **8** in 92% yield after purification (Scheme 2). The orthogonality of this process is demonstrated by the ability to employ both the hydroxy and the acetonide terminated bis-MPA dendrons with no unwanted side reactions occurring at the numerous chain ends. The efficiency of this polymer coupling reaction was further proved by GPC, NMR and MALDI analysis, the latter showing a single peak at 1985 (MH$^+$) for **8**.[11] Using the same methodology, a series of amphiphilic dendrimers from generation 1 to 4 were prepared from dendrons of varied sizes. For example, the asymmetrical structure **9**, in which both the size and functionality of the dendron is varied, was assembled from a hydroxy functionalized [G-1]-azide and an acetonide functionalized [G-4]-acetylene.

The modular nature of this synthetic strategy and the chemical stability of triazoles, azides and acetylenes allows for the efficient introduction of functional groups at different stages of the process. For example, the asymmetrical dendrimer (An)$_{16}$-[G-4]-[G-1]-(OH)$_2$, **9**, was first decorated with alkyne groups by esterification of the two free hydroxyls with pent-4-ynoic anhydride (Scheme 3). Removal of the acetonide protecting groups on the 4th generation dendritic block of **10** revealed 16 reactive hydroxy groups (**11**) followed by attachment of the two 7-diethylaminocoumarin dyes[12]
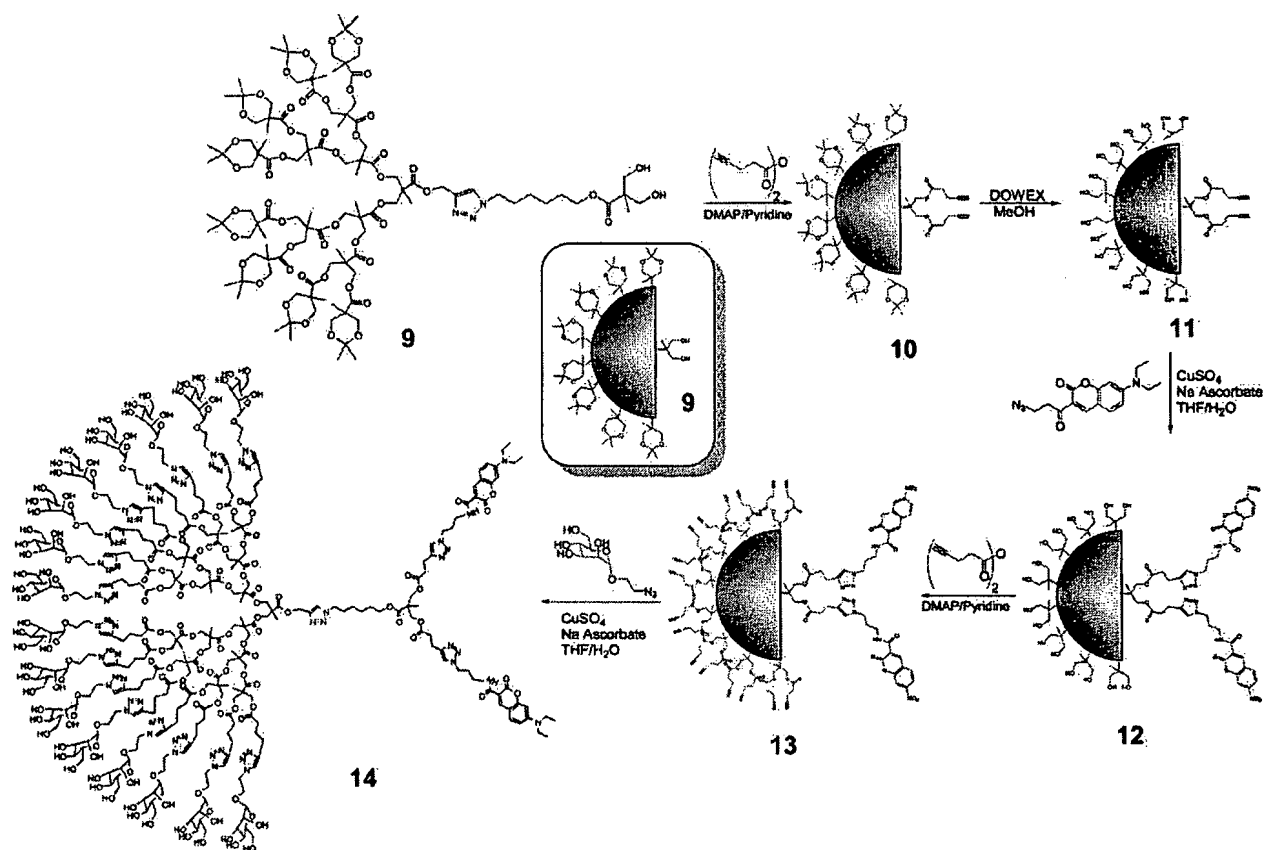
Scheme 3 Synthesis of multivalent, asymmetrical dendrimer **14** containing 16 mannose units and 2 coumarin chromophores.
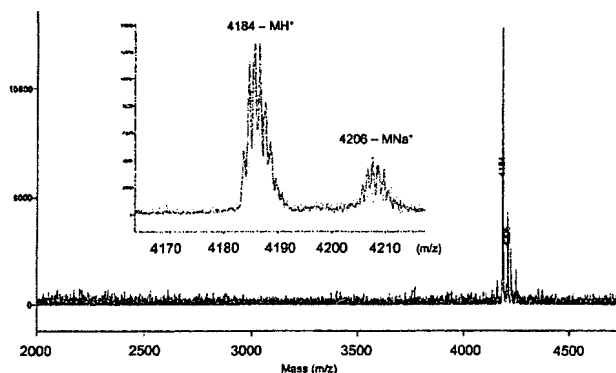
Fig. 1 MALDI mass spectrum of the differentiated, dye-labelled dendrimer 13 showing efficient functionalization and monodispersity.

to the alkyne units resulting in **12**. After introduction of the 16 alkynes (via esterification with the anhydride of pentynoic acid), the resulting dendrimer **13** was directly coupled with the unprotected 2-azidoethyl α-D-mannopyranoside in THF–water to furnish the desired asymmetrical, dual functionalized dendrimer, **14**. Complete characterization of the asymmetric dendrimers by GPC, NMR and MALDI spectroscopy showed essentially monodisperse materials with quantitative functionalization of the chain ends after every step. For example, the acetylene functionalized, fluorescently labelled dendrimer **13** showed a single molecular ion (MH$^+$ = 4184; MNa$^+$ = 4206) in the MALDI spectrum which correlates with 2 coumarin and 16 acetylene chain end groups (Fig. 1). Designed to bear peripheral groups for polyvalent binding (mannose) and fluorescent dyes (coumarin) for visualization/diagnostic purposes, this macromolecular structure is an example of the sophisticated, multifunctional nanomaterials that can be constructed in a stepwise, yet facile manner using Click methodology.[13]

The performance of the mannosylated dendrimer was evaluated in the standard hemagglutination assay using the mannose binding protein concanvalin A and rabbit red blood cells.[14] Dendrimer **14** exhibited 240-fold greater potency than monomeric mannose, corresponding to a relative activity of 15 per sugar moiety when compared to mannose (activity = 1). This demonstrates the synergistic benefit provided by the multivalent, dendritic array of receptor groups. A complete study of polyvalent affinity vs. dendrimer size and generation number is underway and will be described in the future.

In summary, copper(I)-catalyzed azide–acetylene cycloaddition[15] has proven to be a powerful tool for both the preparation of unsymmetric diblock dendrimers and for efficient differentiation of the dendritic chain end groups. By preparing dendrons with unique acetylenic and azide groups at the focal point, highly efficient coupling of these blocks was achieved through the formation of a stable [1,2,3]-triazole linkage. Quantitative modification and sequential differentiation of the chain ends by

the introduction of mannose and coumarin unit derivatives to the periphery of individual blocks allowed preparation of agents with dual function, recognition and detection, which may prove useful in identification and treatment of pathological conditions via multivalent interactions.

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Cu^I-Catalyzed Alkyne–Azide "Click" Cycloadditions from a Mechanistic and Synthetic Perspective

Victoria D. Bock,[a] Henk Hiemstra,[a] and Jan H. van Maarseveen*[a]

Cu^I-catalyzed alkyne–azide cycloaddition provides 1,4-disubstituted 1,2,3-triazoles with such efficiency and scope that the transformation has been described as "click" chemistry. An overview of the mechanism of this remarkable reaction is presented as a means to explain the myriad of experimental results, particularly the various methods of catalyst generation, solvent and substrate effects, and choice of base or ligand. Both solution-phase and solid-phase results are comprehensively examined.

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1. Introduction to the Cu^I-Catalyzed Alkyne–Azide "Click" Cycloaddition

Although demand for new chemical materials and biologically active molecules continues to grow, chemists have hardly begun to explore the vast pool of potentially active compounds.[1] The emerging field of "click chemistry," a newly identified classification for a set of powerful and selective reactions that form heteroatom links, offers a unique approach to this problem.[2] Reactions defined as "click" reactions require only benign reaction conditions and simple workup and purification procedures and can still rapidly create molecular diversity through the use of reactive mod-

ular building blocks.[2] By focusing the search for new compounds only on those available through these reliable and efficient reactions, click chemistry may accelerate the process of discovery and optimization.

Sharpless and co-workers have identified a number of reactions that meet the criteria for click chemistry,[2] arguably the most powerful of which discovered to date is the Cu^I-catalyzed variant of the Huisgen 1,3-dipolar cycloaddition[3] of azides and alkynes to afford 1,2,3-triazoles. As is not uncommon in organic synthesis, this reaction owes its usefulness in part to the ease with which azides and alkynes can be introduced into a molecule and their relative stability under a variety of conditions. Azides and alkynes are essentially inert to most biological and organic conditions, including highly functionalized biological molecules, molecular oxygen, water, and the majority of common reaction conditions in organic synthesis.[4,5] In most cases, the

[a] Van 't Hoff Institute for Molecular Sciences, University of Amsterdam,
Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands

Jan van Maarseveen was born in Enschede, The Netherlands, in 1963. He studied chemistry at the University of Nijmegen and received his Ph. D. at this university with Prof. Binne Zwanenburg and Dr. Hans W. Scheeren in 1994. In the same year, he joined Solvay-Pharmaceuticals (Weesp, The Netherlands) as a group leader in the Medicinal Chemistry Department. He was appointed associate professor at the University of Amsterdam in 1999. In 2004 he joined the Ghadiri group at The Scripps Research Institute (La Jolla, USA) briefly to develop new methods for cyclic peptide synthesis using click chemistry. His current research interests are the development of novel synthetic methodology to enable difficult peptide cyclizations, homogeneous catalysis and the combination of organic synthesis and biology.

Henk Hiemstra was born in Dronrijp, Friesland, The Netherlands, in 1952. He studied chemistry at the University of Groningen and received his PhD. at this university with Prof. Hans Wynberg in 1980. After a postdoctoral stay with Prof. Barry M. Trost at the University of Wisconsin, Madison, USA, he was appointed at the University of Amsterdam in 1982. He was promoted to full professor of organic synthesis in 1997. His favorite research areas are new synthetic methodology and the total synthesis of natural products.

Victoria Bock was born in Webster, Texas, USA, in 1981. She received her B. A. in chemistry from Williams College (Williamstown, Massachusetts, USA) in 2004. She is currently studying at the University of Amsterdam in an international MSc. program.

MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

two functionalities can be installed when convenient and remain unaffected through a number of subsequent transformations.[6] In particular, despite the thermodynamic favorability of azide decomposition, kinetic factors allow aliphatic azides to remain nearly invisible until presented with a good dipolarophile.[5]

In fact, this kinetic stability of alkynes and azides is directly responsible for their slow cycloaddition, which generally requires elevated temperatures and long reaction times.[7,8] Good regioselectivity in the uncatalyzed Huisgen-type cycloaddition is observed for coupling reactions involving highly electron-deficient terminal alkynes,[9] but reactions with other alkynes usually afford mixtures of the 1,4- and 1,5-regioisomers (Scheme 1).[7]
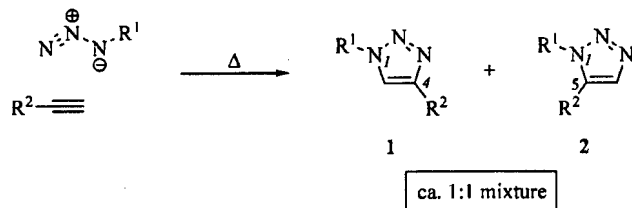
Scheme 1. Products of thermal 1,3-cycloaddition.

Thus, only following the recent discovery of the advantages of Cu^I-catalyzed alkyne–azide coupling, reported independently by the Sharpless[5] and Meldal[10] groups, did the main benefits of this cycloaddition become clear. Cu^I catalysis dramatically improves regioselectivity to afford the 1,4-regioisomer exclusively (1, Scheme 1) and increases the reaction rate up to 10^7 times,[11] eliminating the need for elevated temperatures. This high-yielding reaction tolerates a variety of functional groups and affords the 1,2,3-triazole product with minimal work-up and purification,[5,10] an ideal click reaction.

Further interest in this reaction stems from the interesting biological activity of 1,2,3-triazoles. These heterocycles function as rigid linking units that can mimic the atom placement and electronic properties of a peptide bond without the same susceptibility to hydrolytic cleavage (Scheme 2).[2b,12] Some structural differences between triazoles and amide bonds of course exist; most notably, the extra atom in the triazole backbone leads to a calculated increase in R^1–R^2 distance of 1.1 Å over the typical amide bond (Scheme 2). Triazoles also possess a much stronger dipole moment than an amide bond,[13] but this may actually enhance peptide bond mimicry by increasing the hydrogen bond donor and acceptor properties of the triazole. In addition to the possibility of both the N(2) and N(3) triazole atoms acting as hydrogen-bond acceptors, the strong dipole may polarize the C(5) proton to such a degree that it can function as a hydrogen-bond donor, like the amide proton.[12b,14] Perhaps due in part to their ability to mimic certain aspects of a peptide bond, many known 1,2,3-triazoles possess varied biological activity, including *anti*-HIV activity,[15] selective β$_3$ adrenergic receptor inhibition,[16] *anti*-bacterial activity,[17] potent *anti*-histamine activity,[18] and more.[19,20]
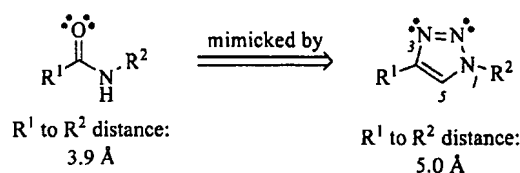
Scheme 2. Topological and electronic similarities of amides and 1,2,3-triazoles.

Since the initial discovery of Cu^I-catalyzed alkyne–azide coupling, numerous successful examples have been recorded in the literature, but as of yet, no systematic study of optimal conditions has been reported. Further, conditions have varied widely, particularly with respect to generation of the active Cu^I species. Sources of Cu^I include Cu^I salts, most commonly copper iodide,[10] in-situ reduction of Cu^{II} salts, particularly Cu^{II} sulfate,[5] and comproportionation of Cu^0 and Cu^{II}.[21] Recent reports suggest that nitrogen-based ligands can stabilize the Cu^I oxidation state under aerobic, aqueous conditions and promote the desired transformation.[22] Steric factors and electronic effects may also play a role in the success of this click chemistry.[10] Herein, we report a mechanism-based approach to unraveling the myriad of results reported since the discovery of Cu^I-catalyzed alkyne–azide coupling.

2. Mechanism of Cu^I-Catalyzed Alkyne–Azide Coupling

2.1 Mechanistic Outline of Cu^I-Catalyzed Alkyne–Azide Coupling

Any mechanism put forth for Cu^I-catalyzed alkyne–azide coupling should explain the body of experimental evidence that makes this transformation so unique. It tolerates most organic functional groups and shows a wide scope with respect to both alkyne and azide reactants. The reaction proceeds in a variety of solvents, tolerates a wide range of pH values, and performs well over a broad temperature range. To this end, researchers at The Scripps Institute in La Jolla, California, USA have proposed a stepwise mechanism on the basis of calculations and kinetic studies (Scheme 3).[21c,23]

Although the thermal dipolar cycloaddition of azides and alkynes occurs through a concerted mechanism, DFT calculations on monomeric copper acetylide complexes indicate that the concerted mechanism is strongly disfavored relative to a stepwise mechanism (Scheme 3). Although one can imagine, for example, direct, concerted cycloaddition of a copper–acetylene π complex with the appropriate azide, the calculated activation barrier for this process exceeds that of the uncatalyzed process, and the lowest barrier found for any concerted process is 23.7 kcal/mol,[21c] too high to be responsible for significant rate effect of Cu^I catalysis. Stepwise cycloaddition catalyzed by a monomeric Cu^I species lowers the activation barrier relative to the uncatalyzed process by as much as 11 kcal/mol, which
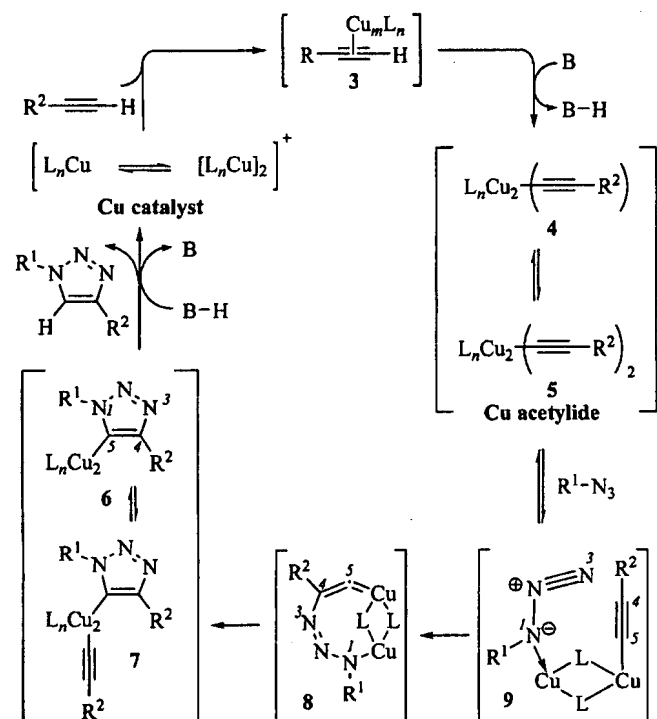
Scheme 3. Proposed outline of species involved in the catalytic cycle.

is sufficient to explain the incredible rate enhancement observed under Cu^I catalysis.[21c]

Based on earlier precedent of Cu^I insertion into terminal alkynes[24] and experimental evidence indicating that internal alkynes show no activity in this reaction,[5,10] researchers propose that the stepwise catalytic cycle begins with formation of a Cu^I acetylide species via the π complex 3 (Scheme 3). Alkyne π complexation requires ligand dissociation and is endothermic in acetonitrile by 0.6 kcal/mol. In aqueous solution, however, the formation of copper species 4 is exothermic by 11.7 kcal/mol, a result consistent with experimental findings of a rate acceleration in water. Calculations also indicate that copper coordination lowers the pK_a of the alkyne C–H by up to 9.8 pH units, thus making deprotonation in aqueous systems possible without the addition of a base.[21c] Although no prior calculations have shown such a significant effect on pK_a, Cu^I acetylides have been found in aqueous solution, even at acidic pH.[25]

Recent kinetic studies indicate that the rate of the catalytic process is second order in copper, but that with increasing copper concentrations, less reactive species such as metal aggregates form.[23] These results suggest that a dynamically changing family of different Cu^I acetylide species may exist in solution, depending on the reaction conditions.[26] Even π complexes may play a role, further complicating matters. While the role of the second copper atom seems to be activation of the azide functionality as in dimer 9 (Scheme 3), π complexation of a terminally bound acetylide to a proximal copper atom may also occur. This complexation doubtlessly changes the reactivity of the acetylide and likely increases acetylide activity toward cyclization by reducing the alkyne electron density.[10,26]

Overall, however, surprisingly little is known about the nature of the copper acetylide complexes active in Cu^I-catalyzed alkyne–azide coupling, even though evidence suggests that these species determine in large part the rate and success of catalysis. Under conditions of excess copper, the reaction was found to be between first and second order in alkyne concentration, suggesting either that two pathways, involving one and two acetylenes, respectively, are operative or that the preferred pathway requires two acetylenes but is inhibited at higher concentrations.[23] In the latter case, higher concentrations of the alkyne may in effect coordinatively saturate the copper ion, due to the Cu^I preference for alkyne ligands over azide ligands, preventing the azide from binding and reducing the overall rate. Commercially available copper acetylides, which are presumably already saturated with alkyne, show no catalytic activity, emphasizing the importance of labile ligand dissociation to catalysis, and as of yet, only one known[27] copper species has been shown to catalyze triazole formation.[23] Although questions about the nature of Cu^I acetylene complexes still exist, the current evidence indicates that the copper acetylide species involved in catalysis requires two metal centers, one or two alkyne ligands, and other labile ligands that allow for competitive azide binding.

Following the formation of the active copper acetylide species, azide displacement of one ligand generates a copper acetylide-azide complex, such as the dicopper species 9 (Scheme 3). Subsequent cyclization has been explored only for monomeric copper species,[21c] but we can imagine a similar process occurring for dimeric copper complexes. Complexation of the azide activates it toward nucleophilic attack of acetylide carbon C(4) at N(3) of the azide (numbers based on traditional triazole nomenclature), generating metallocycle 8.[28] Consistent with this mechanism, experimental results indicate that electron-withdrawing substituents on the alkyne accelerate Cu^I-catalyzed alkyne–azide coupling.[10,26b] This metallocycle positions the bound azide properly for subsequent ring contraction by a transannular association of the N(1) lone pair of electrons with the C(5)–Cu π* orbital. Calculations indicate that ring contraction in the monomeric case ensues from the metallocycle intermediate with almost no barrier;[21c] the difference in ring size for dimeric complexes may change the kinetics slightly, but most likely the transformation from metallocycle 8 into triazole-copper derivative 6 is similarly fast.

Protonation of triazole-copper derivative 7 followed by dissociation of the product ends the reaction and regenerates the catalyst (Scheme 3). Limited deuteration studies suggest that protonation occurs through interaction with a protonated external base or solvent molecule,[21c,23] but further studies are needed to conclusively establish the proton source.

2.2 Mechanistic Studies

Although kinetic and computational studies provide this broad mechanistic outline, recent unexpected experimental

results add more questions into the precise nature of the reactive intermediates. In particular, the role of π complexation in the catalytically-active species remains uncertain. Finn and co-workers evoke π complexation as a means of positioning substrates to explain the unusual cyclodimerization observed during an attempted cyclization of a peptide on the solid phase, but other factors such as influence from the resin and copper–peptide interactions may also contribute to the anomalous results.[29]

Further, π complexation may also activate a terminally-bound copper acetylene toward cycloaddition. Such activation may help explain unusual product distributions observed for various diazides and dialkynes subjected to Cu^I-catalyzed alkyne–azide coupling.[23] Reaction of diazide **10** with phenylacetylene affords the ditriazole **12** as the major product (Scheme 4), even under conditions of excess diazide, while reaction of the analogous dialkyne **19** with benzyl azide provides a statistical mixture of the monotriazole **20** and the ditriazole **21** (Scheme 5) Kinetic studies indicate that during the cycloaddition of the diazide **10**, a low level of monotriazole **11** forms and remains constant throughout the reaction, while the monotriazole **20** builds up initially before leveling off in a manner consistent with two sequential reactions of approximately equal rates.
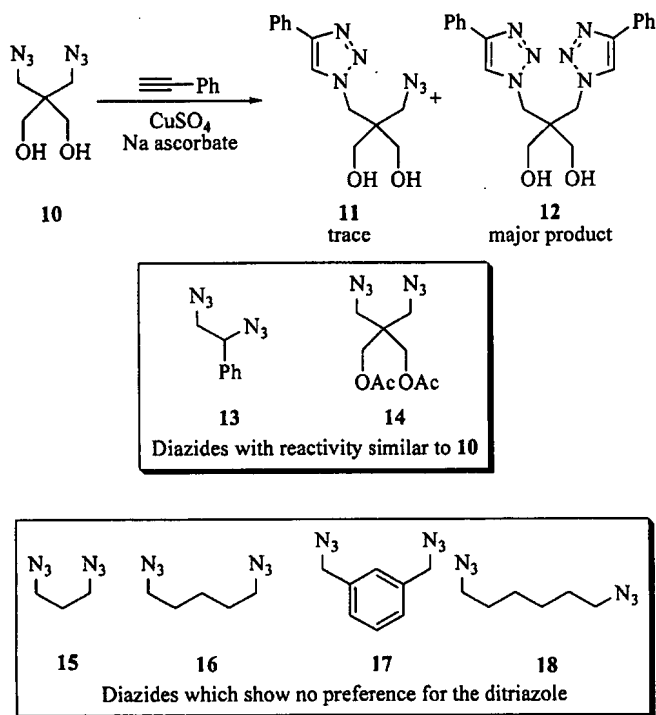
Scheme 4. Reactivity of diazides toward Cu^I-catalyzed cycloaddition.

Only conformational constrained diazides such as **10, 13,** and **14** show this activity (Scheme 4), indicating that the first triazole must be held in close proximity to the azide for this effect to occur.[23] Reactions of diazide **10** show no evidence of autocatalysis, and no rate acceleration was observed in the coupling of phenylacetylene and benzyl azide upon addition of the ditriazole **12**. These results suggest
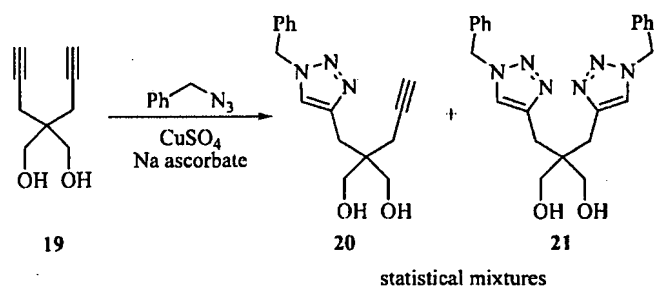
Scheme 5. Cu-catalyzed cyclization of dialkyne **19**.

that the formation of the first triazole catalyzes the subsequent cycloaddition to give the ditriazole. Cu^I-catalyzed alkyne–azide cycloaddition of the independently-prepared monotriazole **11** proceeded at a higher rate than cycloaddition of the diazide **10**, but the rate acceleration was not sufficient to explain the overwhelming preference for ditriazole **12**. Based on these findings and results indicating that the conversion of diazide **10** into ditriazole **12** occurs via some intermediate other than monotriazole **11**, Finn and co-workers propose a mechanism based on capture of intermediate **22** before protonation that would yield free ditriazole **12** (Scheme 6).[23]

Initial cycloaddition yields the copper triazole intermediate **22**, via the same mechanism outlined for the formation of the copper triazoles **6** and **7** (Scheme 3). This intermediate can either undergo protonation to afford monotriazole **11** or can associate with another terminal alkyne[30] or a copper acetylide species[30a,31] to give intermediates **23** and **24**, respectively. Due to the favorable conformation that holds the alkyne and azide functionalities in close proximity in both intermediates **23** and **24**, rapid intramolecular triazole formation is expected from either intermediate.[23] Based on kinetic results indicating the presence of two copper ions in the active copper acetylide species, however, the reaction more likely proceeds via copper dimer **24**. Intermediate copper monotriazole **22** is a simplification of the actual complex, which most likely contains two copper ions and perhaps a bound acetylene (see triazole **6**, Scheme 3). Ligand dissociation could facilely lead to copper dimer **24**, which is not only properly positioned for rapid intramolecular cyclization, but also has an activating π-interaction between the triazole-bound copper ion and the acetylene. Additionally, formation of triazole-copper complex **25** explains the rate enhancement of monotriazole **11** over diazide **10** by directing the Cu^I acetylide to the azide. As a side-note, these results also suggest that copper–triazole intermediates **6** or **7** (Scheme 3) may have significant lifetimes, even in aqueous solution, since capture by an alkyne or copper acetylide species seems to occur more quickly than protonation.[32]

Overall, kinetic and mechanistic studies have established the presence of two copper ions, one or two acetylenes, and one azide in the active complex. π complexation of a copper acetylide species to another copper ion may help position substrates appropriately for cycloaddition and activate the acetylene for attack by the azide. Further studies into the
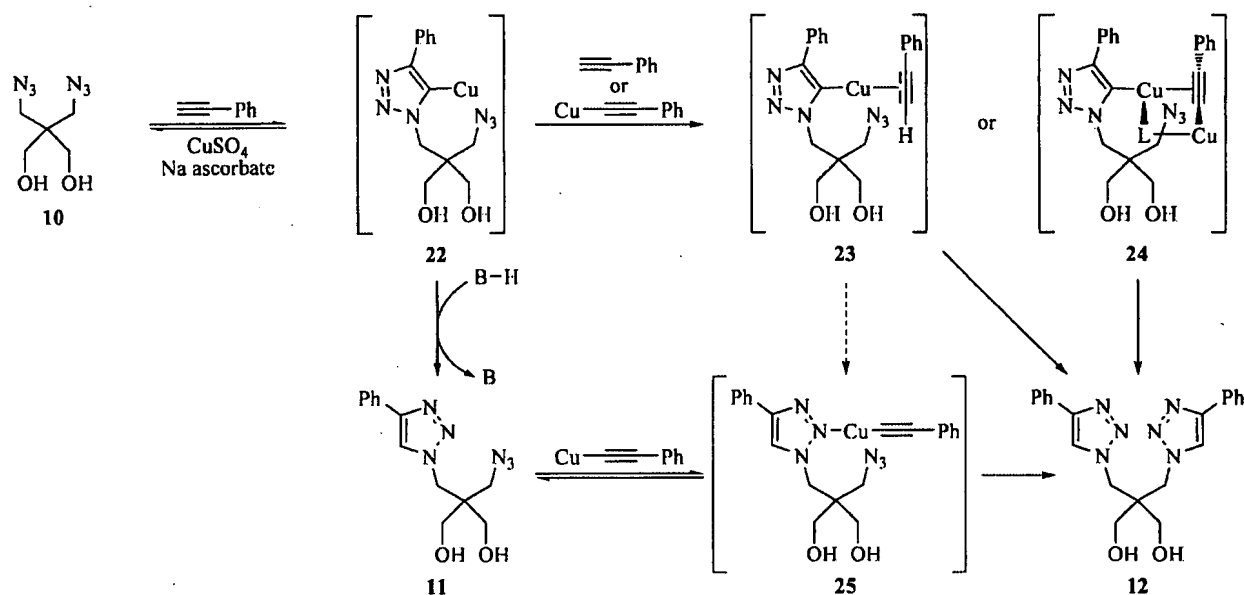
Scheme 6. Proposed mechanism to account for diazide reactivity.

mechanism of Cu^I-catalyzed alkyne–azide coupling should reveal more of the character of the active copper acetylide species, but to date, its exact nature remains unknown.

3. Analysis of Results Reported for Cu^I-Catalyzed Alkyne–Azide Cycloaddition

3.1 Solution-Phase Results

Since the independent discovery of Cu^I-catalyzed alkyne–azide coupling by two different groups in 2002,[5,10] reported conditions have varied widely, with catalyst generation a particular source of contention. Meldal and co-workers initially described the use of Cu^I salts on the solid phase,[5] while Sharpless and co-workers reported solution-phase in-situ reduction of Cu^{II} salts or comproportionation of Cu^0 and Cu^{II}.[10] No comprehensive studies on the ideal conditions for Cu^I-catalyzed alkyne–azide coupling have been completed to date, but results over the past two years suggest that alkyne–azide coupling affords most triazoles in high yield under a variety of conditions, underscoring the robustness of this reaction.

3.1.1 Formation of Cu^I Catalyst by Reduction of Cu^{II} Salts

In-situ generation of Cu^I from Cu^{II} salts, usually Cu^{II} sulfate pentahydrate, can occur by comproportionation with copper metal or by reduction and has the advantage of not requiring inert atmospheres despite the instability of the Cu^I oxidation state in the presence of oxygen.[33,34] Although both reduction and comproportionation have a wide scope and tolerate many organic functional groups, copper–metal comproportionation is generally limited to special applications, such as biological systems, that preclude the use of most reducing agents.[35] This preference for reduction likely results from a combination of the longer reaction times required for comproportionation and the

simpler workup of the reduction method. In traditional solution chemistry with copper sulfate (Table 1, Table 2),[5,23,36] sodium ascorbate[37] at a three- to ten-fold excess over the copper catalyst is the favored reducing agent, though the use of tris(2-carboxyethyl)phosphane hydrochloride (TCEP)[38] has been reported in biological systems.[35c,39]

Current examples in literature demonstrate the scope of this click reaction and the variety of conditions that have proven successful. Alkyne–azide cycloaddition tolerates a wide range of functional groups, including unprotected alcohols, carboxylic acids, and amines, and shows little sensitivity to steric factors, as even tertiary azides undergo coupling in high yield (see Table 1, Table 2). Most commonly, the reaction is performed in a water/alcohol mixture, which facilitates solvation of lipophilic reactants while still retaining the advantages of water, such as faster reaction times and preclusion of the need for an added base. Mixtures of water and organic cosolvents such as DMSO seem to produce equally good results.[36c] Catalyst loading can be quite low, though molecules with solubility problems in water/alcohol mixtures, such as calixarene **26**, may require a higher loading to obtain a good yield (Table 2).

As expected for a reaction classified as "click chemistry," triazoles are generated for the most part with high yields and almost no workup. Workup for most reactions involves little more than filtration to collect a pure precipitate; even the aqueous extraction required for non-solid product triazoles necessitates only the most basic techniques.[23,36a–36b]

3.1.2 Formation of Cu^I Catalyst by Oxidation of Cu Metal

Oxidation of copper metal provides another method for generating the Cu^I catalyst for triazole formation. Addition of excess copper turnings to azides and alkynes in water/alcohol mixtures affords the corresponding triazoles in good yield (Table 3).[21c] Since reactions with copper turnings require longer running times and more copper than

Table 1. Representative results of solution-phase triazole formation by $CuSO_4$ reduction. All reactions were carried at room temperature and were complete in 12 to 24 hours.

Entry	Product Triazole	Alkyne	Azide	Cu[II] Salt[a]	Na Ascorbate	Solvent	Yield[b]
1[c]		1 eq	1 eq	0.01 eq	0.1 eq	1:1 H_2O:tBuOH	92%
2[c]		1 eq[d]	1 eq	0.01 eq	0.1 eq	1:1 H_2O:tBuOH	93%
3[c]		1 eq	1 eq	0.01 eq	0.1 eq	1:1 H_2O:tBuOH	84%
4[c]		1 eq	1 eq	0.01 eq	0.1 eq	1:1 H_2O:tBuOH	88%
5[c]		1 eq	1 eq	0.01 eq	0.1 eq	1:1 H_2O:tBuOH	88%
6[c]		1 eq	1 eq	0.01 eq	0.1 eq	1:1 H_2O:tBuOH	90%
7[c]		1 eq	1 eq	0.01 eq	0.1 eq	1:1 H_2O:tBuOH	94%
8[e]		2 eq	1 eq	0.01 eq	0.1 eq	1:1 H_2O:EtOH	89%
9[f]		1 eq	1 eq	0.05 eq	0.2 eq	1:1 H_2O:EtOH	86%
10[g]		1 eq	1 eq	0.2 eq $Cu(OAc)_2$	0.4 eq	1:1 H_2O:tBuOH	70%
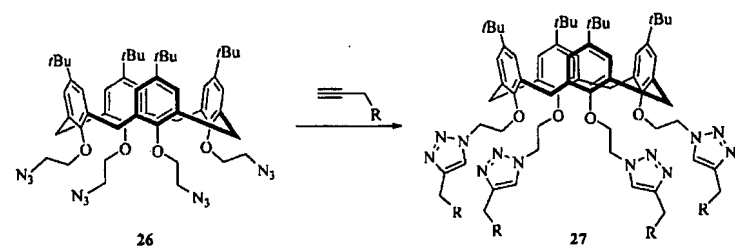
[a] Cu[II] salt is $CuSO_4$ unless otherwise noted. [b] Isolated yield. [c] Ref.[5] [d] Equivalents per azide unit. [e] Ref.[36b] [f] Ref.[36d]. [g] Ref.[36i]

other protocols, nanosize Cu^0 provides an alternative method of catalyst generation comparable to the efficiency of other protocols.[40] Oxidative dissolution of Cu^0 nanosize activated powder into Cu^I by an amine hydrochloride salt[41] yields the desired triazole in good yield (Scheme 7). This protocol shows high tolerance to a variety of functional groups but requires the presence of an amine salt, either added (as in Scheme 7) or incorporated into alkyne or azide.[40a] Presumably, amine hydrochloride salt mediates dissolution of Cu^0 into Cu^I. Subsequent coordination of a nitrogen-based ligand to the Cu^I species then occurs, followed by displacement by an alkyne to give the active copper acetylide species. Cu^I can then either undergo dispro-

portionation[42] to Cu^{II} and Cu^0 or further oxidation to Cu^{II}; oxidation to Cu^{II} apparently prevails, indicated by the blue color that forms upon completion of the reaction.[40a]

Cu^0 nanosize clusters also effectively catalyze alkyne-azide cycloaddition, though without the need for an amine hydrochloride salt (Scheme 8).[40b] Evidence indicates that the reaction takes place on the surface of the nanoclusters rather than in solution, but Cu^I is most likely still the active oxidation state in this reaction.[43]

Reactions with nanosize Cu^0 appear to have the same wide scope and high yields as expected in click chemistry, though the main disadvantages will likely limit future implementation. Solvation of Cu^0 nanosize particles requires
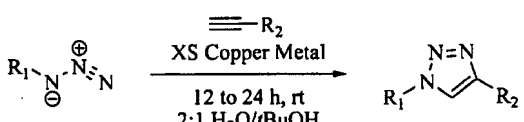
Table 2. Results of solution-phase triazole formation by CuSO$_4$ reduction on calixarenes (data taken from ref.[36h]). All reactions were carried at under N$_2$ at 60 °C and were complete 24 hours.

Entry	Alkyne Substituent	Alkyne	CuSO$_4$	Azide	Na Ascorbate	Solvent	Yield[a]
1	R = (structure with O, OH, OH, OH, OH, OH, NH)	1.25 eq[b]	0.2 eq	1 eq	1.25 eq	1:2:2 THF:H$_2$O:EtOH	51%
2	R = —SO$_3$Na	1.05 eq[b]	0.4 eq	1 eq	4.5 eq	1:2:2 THF:H$_2$O:EtOH	79%
3	R = —NMe$_3$Br	1.05 eq[b]	0.4 eq	1 eq	4.5 eq	1:2:2 THF:H$_2$O:EtOH	78%

[a] Isolated yield. [b] Equivalents per azide unit.

Table 3. Results of solution-phase triazole synthesis with Cu metal (data taken from ref.[21c]).

Entry	Product Triazole	Alkyne	Azide	Yield
1	(structure)	1 eq[a]	1 eq	98%
2	(structure)	1 eq	1 eq	88%

[a] Equivalents per azide unit.
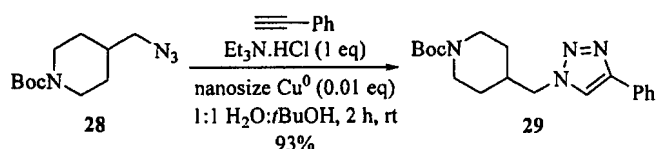
Scheme 7. Typical example of solution-phase triazole synthesis with nanosize Cu^0.

Yields: 80% to >99%

R^1 = C$_6$H$_5$; C$_6$H$_5$CH$_2$-
R^2 = C$_6$H$_5$; C$_6$H$_5$(OH)CH–; CH$_2$(OH)–
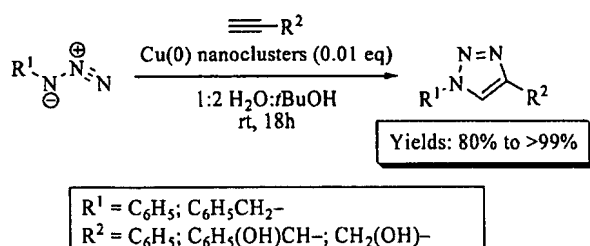
Scheme 8. Triazole formation catalyzed by Cu^0 nanoclusters.

a slightly acidic environment, approximately pH 5, implying that acid-sensitive functional groups that easily withstand other protocols require protection prior to cyclization. Further, Cu^0 nanosize clusters are not commercially available, and Cu^0 nanosize powder costs more than seven times as much as other copper sources utilized in Cu^I-catalyzed alkyne–azide coupling,[44] greatly limiting the applicability of these protocols in research and industry.

3.1.3 Formation of Cu^I Catalyst by Addition of a Cu^I Salt

Initial solution-phase Cu^I-catalyzed alkyne–azide coupling reactions lacked the apparent robustness observed with copper sulfate reduction or Cu^0 oxidation, due to complicating side reactions.[10,45] An early study by Wong and co-

Table 4. Results of cycloaddition of the azide 30 and alkyne 31 (data taken from ref.[46]).
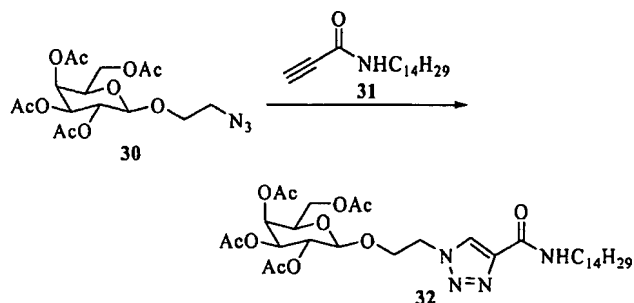
Entry	Alkyne	Base (1 eq)	CuI	Solvent	T [°C]	Time	Yield[a]
1	5 eq	None	None	None	80	24 h	89%[b]
2	1 eq	Et$_3$N	2 eq	MeCN	rt	18 h	trace
3	1 eq	DIPEA	2 eq	MeCN	rt	18 h	38%
4	1 eq	Et$_3$N	0.1 eq	toluene	rt	18 h	65%
5	1 eq	DIPEA	0.1 eq	toluene	rt	18 h	85%
6	1 eq	None	0.1 eq	toluene	rt	3 days	52%
7	1 eq	None	0.1 eq	toluene	rt	7 days	61%

[a] Isolated yields. [b] 4:1 Mixture of 1,4- and 1,5-regioisomers.

workers demonstrated this extreme sensitivity to reaction conditions (Table 4): Triethylamine in acetonitrile afforded no product, whereas use of DIPEA for the base produced the triazole **32** in 38% yield.[46] With no base added (Table 4, Entries 6, 7), reactions proceeded much more slowly, most likely due to difficulties forming the active copper acetylide complex. As discussed above, deprotonation of the π complex **2** to form the copper acetylide **4** (Scheme 3) can occur without the addition of a base in water, but in organic solvents, the formation of **4** is unfavorable and a base is required for deprotonation.[21c]

In practice, bases such as DIPEA and 2,6-lutidine improve results in Cu[I]-catalyzed alkyne–azide coupling by minimizing side-product formation,[5,12,45] but the absence of thorough studies comparing conditions makes comprehensive analysis difficult (Table 5).[47] Excess base appears to give particularly high yields (Table 5, Entry 2), perhaps by stabilizing the Cu[I] oxidation state. Nitrogen-type donors,

including bases and certain solvents such as acetonitrile,[48] help to prevent degradation of Cu[I] by oxidation or disproportionation, which greatly increases reaction rates, especially due to the second-order dependence on Cu[I] concentration.

In general, a variety of triazole products are obtained in good yield through a range of conditions. Fluoro-substituted triazoles, however, prove more difficult to synthesize (Table 5, Entry 6),[47f] likely due to their highly electron-deficient nature. Binding to the copper–acetylene complex may be less favorable, enabling more side-product formation to occur.[45] Other factors may be involved, however, as unprotected alcohols interfere with cycloaddition of fluoro-substituted azides,[47f] a problem not observed with other systems.[49]

Cu[I] salts therefore represent a reliable means of catalyzing alkyne–azide cycloaddition, particularly in the presence of excess base. Optimal solvent and base conditions may

Table 5. Results of solution-phase triazole formation by Cu[I] salt addition. All reactions were carried at room temperature.

Entry	Product Triazole	Alkyne	Azide	Cu Salt	Base	Solvent	Time	Yield[a]
1[b]		1.6 eq	1 eq	0.1 eq CuI	1.1 eq 2,6-lutidine	MeCN	12 h	80%
2[c]		1 eq	1 eq	0.1 eq CuI	2 eq (each) 2,6-lutidine DIPEA	MeCN	3 h	97%
3a[d] R¹ = H, R² = OBnl		1 eq	1 eq	0.1 eq CuI	1 eq DIPEA	toluene	15 h	80%
3b[d] R¹ = OBnl, R² = H								82%
4a[e] R = H		1 eq	1 eq	0.1 eq CuI	1 eq DIPEA	toluene	18 h	85%
4b[e] R = Ac								80%
5[f]		1.5 eq	1 eq	0.13 eq CuI	2 eq Et₃N	1:1 H₂O/MeOH	1 h	63%
6a[g] R = Ph								66%
6b[g] R = C₆H₁₃		1.1 eq	1 eq	0.01 eq CuI	1.1 eq Et₃N	2:1 H₂O/MeCN	20 h	77%
6c[g] R = CO₂Me								37%

[a] Isolated yield. [b] Ref.[47a], dry conditions. [c] Ref.[12a], dry conditions, inert atmosphere. [d] Ref.[47b] [e] Ref.[46] [f] Ref.[47d] [g] Ref.[47f]

depend on the substrate, but in general, this reaction shows good results under varying conditions.

3.1.4 Ligand-Assisted Cu^I-Catalyzed Alkyne–Azide Coupling

Although alkyne–azide cycloaddition is effectively catalyzed under "ligand-free" conditions in which only solvent molecules or bases serve as ligands to the metal, the addition of certain heterocyclic chelates further accelerates the reaction, likely by shielding the Cu^I ion from interactions leading to degradation.[22] As a result of this, ligands such as amine triazole 33 dramatically reduce the minimum catalyst loading as much as tenfold without requiring longer reaction times (Table 6). Further, addition of ligand 33 precludes the need for base even in organic solvents, as the tertiary nitrogen center functions both as a donor to Cu^I and a proton acceptor (Table 6, Entry 1).

Table 6. Ligand-assisted Cu^I-catalyzed cycloaddition.

Entry	Conditions	Yield
1[a]	Cu(MeCN)$_4$PF$_6$ (0.01 eq), ligand 33 (0.01 eq) 2:1 tBuOH/H$_2$O, rt, 24 h	84%
2[b]	Na ascorbate (0.05 eq), CuSO$_4$ (0.0025 eq) ligand 33 (0.005 eq), 1:1 EtOH/H$_2$O, 4 h, rt	> 85%

[a] Ref.[22]. [b] Ref.[50].

In contrast to the limited research into ligand effects in traditional solution-phase chemistry, the numerous examples of ligand rate acceleration in bioconjugation provide additional evidence of the stabilizing properties of ligand 33.[35,39,50] In fact, one study investigating in-vivo proteome labeling observed that Cu^{II} sulfate and ligand 33 efficiently catalyzed triazole formation, even in the absence of a reducing agent.[39a] Interestingly, however, copper catalysis lacking both a reducing agent and ligand 33 afforded significantly reduced levels of labeled product, suggesting that the endogenous reductant produces only a low concentration of Cu^I that requires ligand stabilization to efficiently produce labeled product. Of the research currently available, only a study utilizing alkyne–azide cycloaddition to couple dyes onto protein observed no rate enhancement or improvement of any sort through addition of ligand 33.[35a]

While amine triazole 33 was first reported as the most efficient ligand for cycloaddition catalysis,[22] various other ligands, including chiral pybox ligands such as pybox ligand 37 (Scheme 9), effectively accelerate cycloaddition as well.[51] These chiral ligands also add enantioselectivity to click chemistry: Fokin, Finn, and co-workers reported modest levels of selectivity in the kinetic resolution of racemic azide 35, with a 2:1 ratio of CuI/37 proving most effective.

Attempted kinetic resolution of azides 38 to 40 proved unsuccessful, with only low rates of selectivity observed,[52] and benzyl acetylenes analogous to azide 35 reacted with no enantioselectivity (Scheme 10).[51]
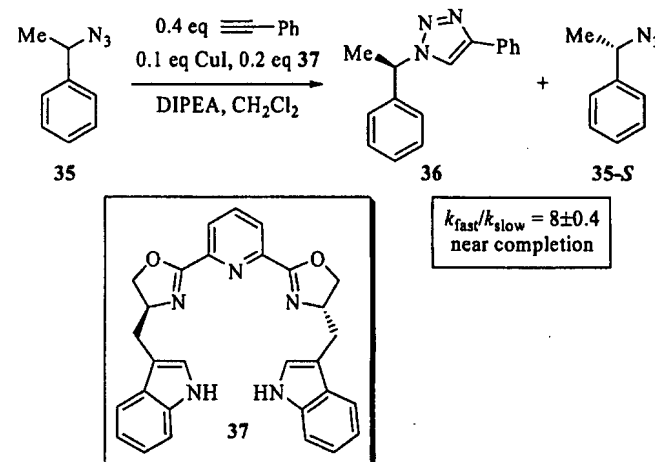
Scheme 9. Kinetic resolution of azide 35.
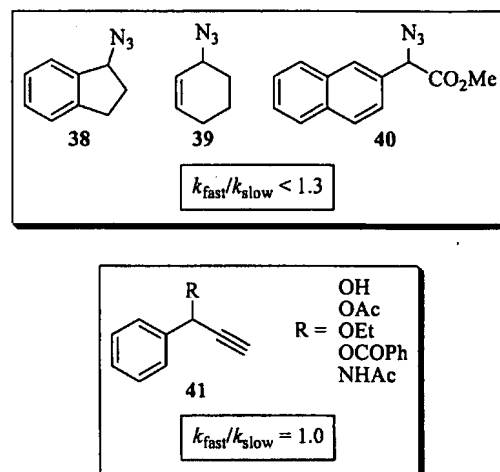
Scheme 10. Results of kinetic resolution on other racemic substrates.

These results suggest that in the acetylene–azide copper intermediate (dimer 9, L$_2$ = 37; Scheme 3), only the azide substituents experience a chiral environment, likely due to proximity to the chiral ligand.[51] As the binding mode of dimer 9 suggests (Scheme 3), the azide substituents lie closer to the Cu^I–ligand complex than the acetylene substituents, resulting in better enantioselectivity for the kinetic resolution of azides. As the precise nature and binding modes of intermediates such as dimer 9 and copper–acetylide intermediates 4 and 5 remains to date unknown (Scheme 3), other factors such as counterion association or complex binding modes may also influence reactivity.

Overall, ligand-accelerated cycloaddition seems to suit bioconjugation studies particularly well, especially in-vivo experiments that may limit the use of other bases or means of improving reaction results. Kinetic resolution is also an interesting application, particularly with respect to mechanistic aspects of alkyne–azide cycloaddition, but for tradi-

tional chemistry, especially on the scale done in most academic laboratories, the necessity of removing the ligand from the subsequent products complicates the simple workup and purification that make this click chemistry so attractive to chemists. While addition of ligands such as **33** or **37** may improve certain aspects of the cycloaddition, other methods, such as use of excess base, may in some cases produce the same results and preclude the need for additional purification.

3.1.5 One-Pot Multi-Step Reactions involving Cu[I]-Catalyzed Alkyne–Azide Coupling

Given the unfavorable reputation of azides among organic chemists, in-situ generation of azides without isolation can further expand the applications of copper catalyzed click chemistry. Such one-pot procedures can help to expand the efficiency and further broaden the scope of this reaction, particularly if the wide scope and reliability of

alkyne–azide coupling is maintained. Gratifyingly, the newly-reported one-pot procedures involving click chemistry show the same level of success as expected from traditional alkyne–azide coupling reactions.

One-pot synthesis of triazole-substituted glycoconjugates from unprotected monosaccharides utilizes click chemistry to rapidly link complex monomers.[53] In one pot, acetate protection, brominolysis, and subsequent azide generation in the presence of an acetylide yields the expected triazole product in good yield over the four transformations (Table 7). Alkyne–azide coupling on protected D-glucose and D-galactose proceeds smoothly overnight at room temperature, but steric interference from the β-substituent on the three position of D-mannose reduces the reaction rate such that elevated temperatures are required to obtain the desired triazole (Table 7, Entries 6b, 7).[54] Wang and co-workers also demonstrate success with in-situ azide generation and subsequent Cu[I]-catalyzed alkyne–azide cycloaddition on saccharides; their methods afford even polyvalent glycoconjugates in fair yield, demonstrating the efficiency of their methodology.[53]

Combining multiple reactions in a single pot requires specific selectivity and minimal side-product formation; the inertness of alkyne and azide functional groups makes alkyne–azide coupling ideal for this purpose. Researchers have recently developed a protocol for a one-pot reaction involving four transformations (Scheme 11): the Wittig olefination, the Knoevenagel condensation, the Diels–Alder cyclization, and Cu[I]-catalyzed alkyne–azide cycloaddition.[55] This reaction works with a variety of organic azides and provides high yields over the four transformations.

Table 7. One-pot synthesis of triazolylglycosides by click chemistry (data taken from ref.[53]). Reactions with glucose and galactose were conducted at room temp. and run overnight; reactions with mannose were conducted at 80 °C and run overnight.

Entry	Sugar Moiety	Alkyne	Yield[a]
1a	D-Glucose	≡—Ph	95%
1b	D-Galactose		71%
2	D-Glucose		81%
3	D-Glucose		77%
4	D-Glucose		71%
5	D-Glucose		72%
6a	D-Galactose		71%
6b	D-Mannose		98%
7	D-Mannose		71%

[a] Isolated yield.

Scheme 11. One-pot Wittig Knoevenagel Diels–Alder click cycloaddition.

3.1.6 Microwave-Assisted Cu[I]-Catalyzed Alkyne–Azide Coupling

Since the first reports in 1986,[56] microwave-assisted organic synthesis has generated considerable attention by producing products in cleaner, higher-yielding reactions than

traditional methodology and by reducing reaction times to minutes and seconds, rather than days and hours.[57] Since the energy of the microwave photons in all current dedicated microwave reactors is too low to break chemical bonds,[58] the enhancement observed in microwave chemistry may result from efficient dielectric heating that creates an inverted temperature gradient relative to traditional heating methodologies.[58c]

Although Cu^I-catalyzed alkyne–azide coupling often requires no additional heating, microwave chemistry can dramatically reduce reactions times in many cases from over twelve hours (see Table 5) to less than one hour (Table 8).[36a,59] Yields for these reactions show no considerable gain over traditional methodology,[60] suggesting that microwave heating increases the rate of the desired reaction and any undesired side reactions equally.[45]

Alkyne substituent effects, similar to those observed for cycloaddition reactions conducted at room temperature, dramatically affect the success of the microwave-assisted reaction. As previously noted for traditional methodologies,[10,26] electron-deficient alkynes react most quickly, likely due to facile formation of the Cu^I acetylide species and increased rate of electrophilic attack by the bound azide (Scheme 3). For reactions utilizing (Ph$_3$P)$_3$CuBr/DIPEA, this effect seems particularly pronounced: Electron-rich alkyne reactants yield no product (Table 8, Entries 2a and 3a) in contrast to the high yield obtained for the more electron-poor oxygen-substituted alkyne (Table 8, Entry 1).[59a] Remarkably, addition of copper iodide to the (Ph$_3$P)$_3$CuBr/DIPEA system (Table 8, Entry 3b) or use of (EtO)$_3$PCuI for (Ph$_3$P)$_3$CuBr (Table 8, Entry 2) dramatically improves the results. Use of DBU, a stronger base than

Table 8. Representative microwave-assisted Cu^I-catalyzed triazole formation. All reactions were carried in a microwave oven.

Entry	Product Triazole	Alkyne	Azide	Cu Source	Base or Reducing Agent	Solvent	Time	Yield[a]
1[b]		1.1 eq	1 eq	0.1 eq (Ph$_3$P)$_3$CuBr	3 eq DIPEA	toluene	34 min	86%
2a[b]		1.1 eq	1 eq	0.1 eq (Ph$_3$P)$_3$CuBr	3 eq DIPEA	toluene	[c]	0%
2b[b]		1.1 eq	1 eq	0.1 eq (EtO)$_3$PCuI	3 eq DIPEA	toluene	15 min	99%
3a[b]		1.1 eq	1 eq	0.1 eq (Ph$_3$P)$_3$CuBr	3 eq DIPEA	toluene	[c]	0%
3b[b]		1.1 eq	1 eq	0.1 eq (Ph$_3$P)$_3$CuBr	3 eq DBU	toluene	46 min	51%
3c[b]		1.1 eq	1 eq	0.1 eq CuI and 0.1 eq (Ph$_3$P)$_3$CuBr	3 eq DIPEA	toluene	26 min	96%
4[b]		1.1 eq[d]	1 eq	0.1 eq (Ph$_3$P)$_3$CuBr	3 eq DIPEA	toluene	11 min	93%
5[e]		1 eq	1 eq	0.02 eq CuSO$_4$	0.2 eq Na ascorbate	DMF	10 min	73%

[a] Isolated yield. [b] Ref.[59a], 850 W, no T reported. [c] No time reported. [d] Equivalents of alkyne per azide functionality. [e] Ref.[59b], 90 °C, no microwave wattage reported.

DIPEA, also offers some improvement over the (Ph₃P)₃-CuBr/DIPEA system for electron-rich alkynes (Table 8, Entry 3a), but the yield remains rather disappointing. DBU likely increases the rate of copper acetylide formation by favoring deprotonation of the π-complexed alkyne (Scheme 3), but the stronger base may also favor side-product formation,[45] resulting in a low yield.

Furthermore, by dramatically increasing reaction rate, microwave-assisted alkyne–azide cycloaddition provides a possible means of overcoming some of the difficulties with aqueous solvents observed with other protocols. For products insoluble in water/alcohol mixtures or too soluble and thus difficult to extract by aqueous workup, microwave-assisted reactions enable rapid product formation in anhydrous organic solvents with extremely low catalyst loadings (Table 8, Entry 5).

Microwave chemistry even facilitates one-pot reactions, generating a variety of triazoles directly from the alkyl halide in under 15 minutes (Table 9).[11] Since this procedure utilizes potentially unstable or volatile organic azides without isolation, novel triazoles inaccessible by the traditional protocol become available (Table 9, Entry 3).

Table 9. One-pot synthesis of selected triazoles by microwave-assisted click chemistry (data taken from ref.[11]).

$$\equiv\!\!-R^2 \xrightarrow[\substack{CuSO_4\ (0.2\ eq),\ Cu(0) \\ 1:1\ tBuOH:H_2O \\ MW\ 100\ W,\ 125\ °C^{[a]}}]{NaN_3\ (1.05\ eq),\ Hal\!-\!R^1\ (1\ eq)} R^1\!\!-\!\!\overset{N=N}{\underset{N}{\diagdown\!\!\diagup}}\!\!-R^2$$

Entry	Halide	Alkyne	Time	Yield
1a Hal = Br	Ph⌢Hal	≡—Ph	10 min	93%
1b Hal = Cl		≡—Ph	10 min	91%
2	Ph⌢⌣Br	≡—Ph	10 min	88%
3	MeI	≡—Ph	10 min	88%
4a R = (CH₂)₂OH			10 min	81%
4b R = C(OH)Me₂			10 min	84%
4c R = CH(OH)C₅H₁₃	Ph⌢Br	≡—R	10 min	89%
4d R = CO₂Et			15 min	83%
4e R = TMS			15 min	88%

[a] Entries 4d and 4e were conducted at 75 °C.

In general, microwave-assisted Cu^I-catalyzed alkyne–azide cycloaddition shares the same wide scope and inertness to functional groups with room-temperature protocols. Nearly all substrates tolerated the high temperatures, which ranged from 75 to 140 °C; for cycloaddition involving ethyl propiolate and (trimethylsilyl)acetylene (Table 9, Entries 4d, 4e), temperatures of 125 °C reduced yields, but the desired products were obtained in good yield at 75 °C without significantly increasing reaction time.[11] These preliminary results suggest that microwave chemistry can dramatically accelerate cycloaddition without affecting yield or ease of product purification,[11,36a,59] further adding to the advantages of Cu^I-catalyzed alkyne–azide coupling.

3.1.7 Cu^I-Catalyzed Alkyne–Azide Coupling in the Solution-Phase Synthesis of Dendritic and Polymeric Materials

Due to the reliability of Cu^I-catalyzed click chemistry, a wide range of complex dendrimers and polymeric materials can be obtained with incredible efficiency, paving the way for applications in nanotechnology and homogeneous catalysis.[36a,36e,61] Interesting highly-branched polymers (45)[61a] and novel conjugated polymers (46)[61b] (Figure 1) form facilely from the corresponding monomers, under CuSO₄/Na ascorbate and Cu(OAc)₂/Cu⁰ conditions, respectively. Coupling of terminal azide-functionalized polystyrene with alkynes also proved successful under conditions of CuBr/(pentamethyl)diethylenetriamine (PMDETA) in THF at 35 °C.[62]
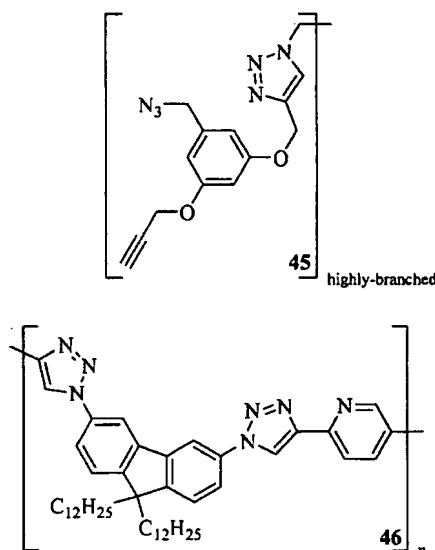
Figure 1. Polymers accessible by click chemistry.

Cycloaddition reactions to form a variety of dendrimers, including fourth-generation dendrimer 47 (Figure 2), utilized CuSO₄/Na ascorbate conditions and gave poly-triazole products in yields over 90%: even the final click reaction to yield dendrimer 47 proceeded in 92% yield.[36a] Dendronized linear polymers, potential new materials for nanoscale applications, are also rapidly accessible via click chemistry (Scheme 12).[36e] Dendrimers as large as third generation underwent facile cycloaddition to poly(vinylacetylene) under CuSO₄/Na ascorbate conditions, but reaction with fourth generation dendritic azides afforded only starting material, likely due to difficulties with azide binding to the copper–acetylide complex.

3.2 Solid-Phase Cu^I-Catalyzed Alkyne–Azide Coupling

Although solution-phase click chemistry has demonstrated the power of this transformation, success on the solid phase makes this cycloaddition an essential tool in drug discovery, which relies on solid-phase combinatorial chemistry to generate new libraries of compounds for biological testing.[2b] Such success transitioning from solution-phase
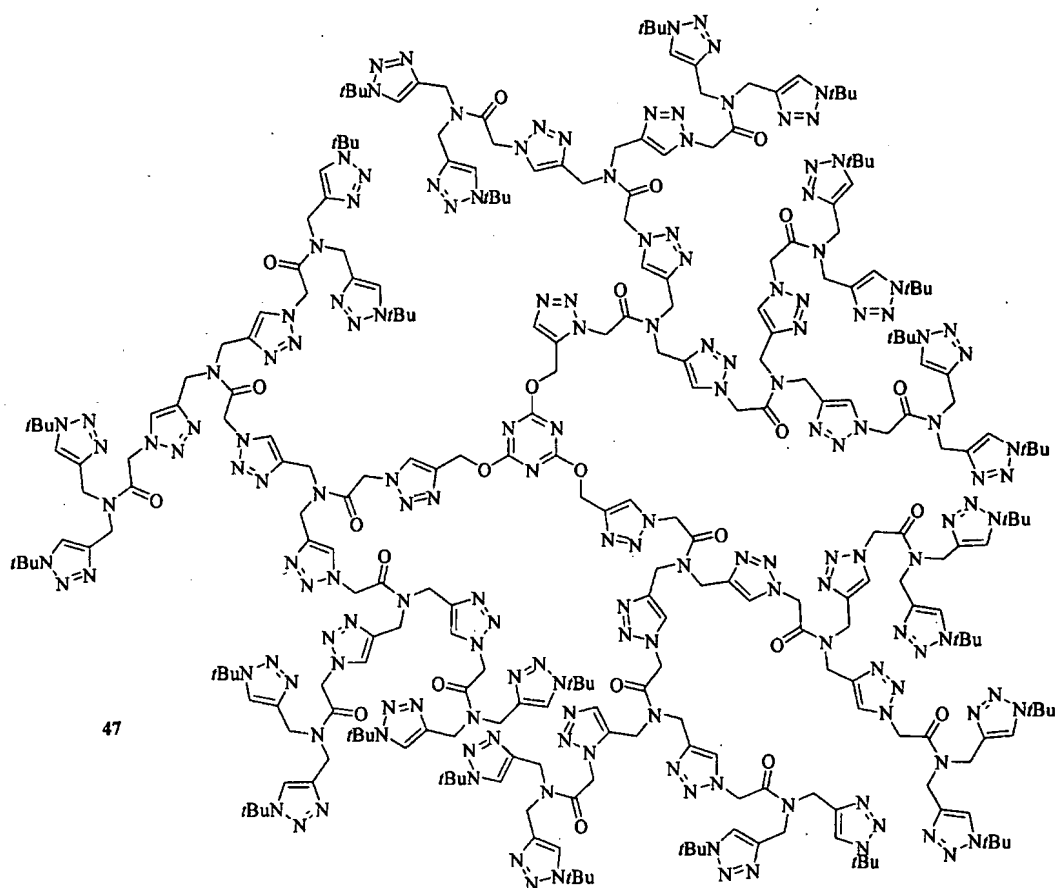
Figure 2. Fourth-generation dendrimer **47**.
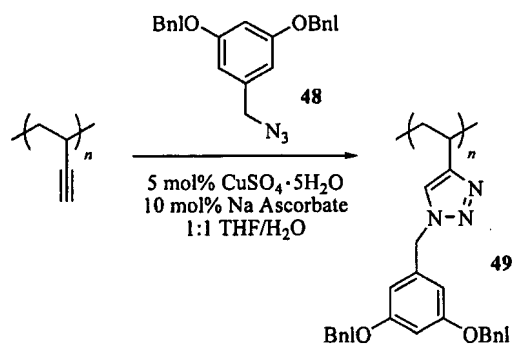
Scheme 12. Synthesis of first-generation-dendronized linear polymers by click chemistry.
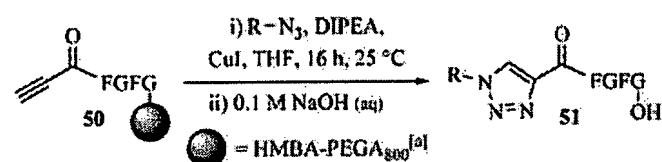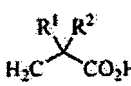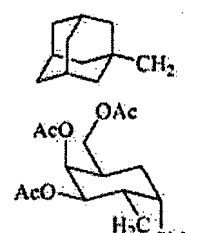
to solid-phase chemistry has made possible further applications of solid-phase cycloaddition, outside the synthesis of biologically interesting compounds; triazoles can serve as linkers to bind other functionalities to solid-phase resins[63] and can functionalize metal surfaces to change their properties[26b] and induce adhesion.[21b]

According to preliminary research results, solid-phase Cu^I-catalyzed alkyne–azide coupling shows little sensitivity to conditions, resin type, or subsequent transformations, though alkyne homocoupling may prove problematic.[45] Both Cu^I salts and in-situ Cu^{II} salt reduction effectively catalyze the reaction[64] in various organic solvents, including THF,[20c,63] DMF,[10] and acetonitrile/DMSO (4:1).[29] Tri-

azole formation on different resins, including polar matrices, such as PEGA, and apolar supports, such as polystyrene, further demonstrates the robustness of solid-phase click chemistry. Thus far, only homocoupling appears to limit the reliability of this chemistry: Meldal and co-workers originally reported difficulty with coupling alkynes onto an azide-substituted resin, due to excessive alkyne homocoupling.[10] More recently, however, triazole formation on azide-substituted resins has proceeded in high yield, even at alkyne concentrations of up to 1.25 M,[63] but for resins with more sterically hindered azide functionalities, alkyne homocoupling may dominate and dramatically reduce yields.

Despite this possible limitation, solid-phase alkyne–azide coupling has potential to generate numerous molecules of diverse functionality (Table 10,[10] Scheme 13[63a]). In general, cycloaddition proceeds facilely on the solid phase but may show more sensitivity to steric issues than solution-phase work (Table 10, Entry 1b). Incorporation of a triazole into a peptide as an amide bond mimic occurs without problem[20c] in the solid phase (Table 10, Entry 2), a particular advantage considering the success of modern peptide synthesis on the solid phase. In addition to triazole-containing products, use of the triazole functionality as a linker to attach substrates to the resin enables the synthesis of any number of functionalities, including non-triazole containing amides (Scheme 13).

Table 10. Purity[b] of selected triazole products formed from resin 50 (data taken from ref.[10]).

Entry	R	Purity[b]
1a $R^1 = R^2 = Me$		>95%
1b $R^1 = R^2 = Ph$		0%
1c $R^1 = R^2 = H$		>95%
1d $R^1 = R^2 = nC_{14}H_{29}$		91%
1e $R^1 = Ph$, $R^2 = H$		>95%
2a[c] R = H		90%
2b[c] R = CH$_2$CH$_2$SMe		84%
3		>95%
4		>95%

[a] HMBA-PEGA$_{800}$: Dimethyl acrylamide and mono-2-acrylamidoprop-1-yl[2-aminoprop-1-yl] polyethylene glycol cross-linked with bis(2-acrylamidoprop-1-yl) polyethyleneglycol (PEGA resin) derivatized with 4-(hydroxymethyl)benzoic acid (HMBA) linker. [b] Conversion was >95% for all reactions except Entry 1b. [c] Fmoc group on primary amine removed after cyclization prior to analysis.

Solid-phase click chemistry is clearly just beginning to develop, but given its reliability and robustness, the potential applications in combinatorial chemistry and materials science will likely increase rapidly.

3.3 Intramolecular Cu^I-Catalyzed Alkyne–Azide Coupling

Despite biological significance of the triazole and the success of intermolecular triazole formation, intramolecular alkyne–azide coupling remains surprisingly limited. On a macromolecular scale, intramolecular triazole formation can occur on specially synthesized DNA molecules,[65] by cyclodimerization of solid-phase-bound suitably functionalized peptides,[29] or on copper surfaces to induce adhesion,[21b] but more traditional examples are rare. Solution-phase chemistry has, to date, only one reported intramolecular triazole cyclization: Successful dimerization followed by intramolecular Cu^I-catalyzed cycloaddition yielded macrocycle 56 in 80% yield (Scheme 14).[66] Interestingly, copper iodide and DBU in toluene proved most efficient of the various copper sources, additives, and solvents explored for this reaction; DBU even gave higher
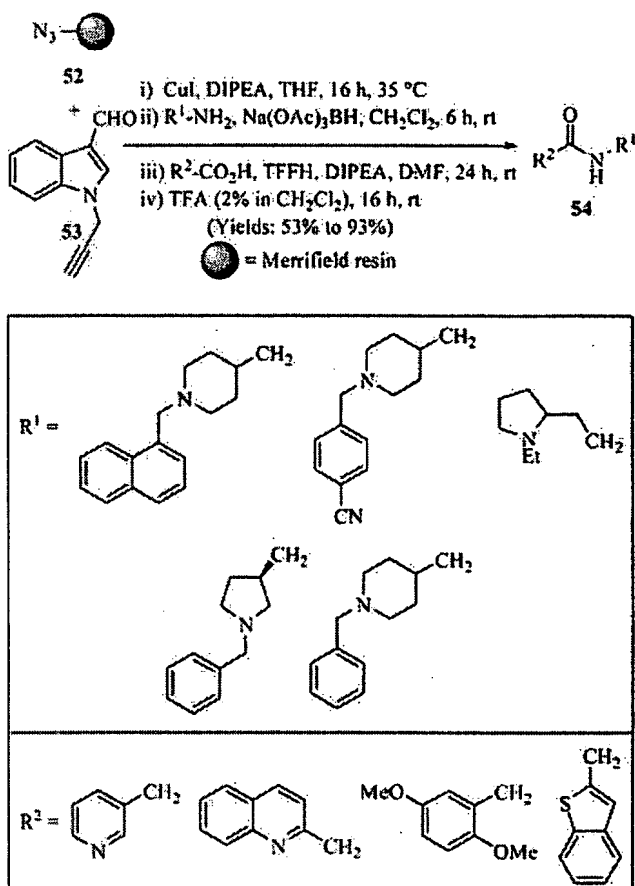
Scheme 13. Amides synthesized by reactions on a triazole-linked resin.

yields than reactions including triazole ligand 33 (Table 6), indicating as before that amide bases may serve to stabilize the Cu^I oxidation state similarly to heterocyclic ligands 33 and 37. Clearly, these conditions succeed in favoring macrocycle 56 over byproduct formation[45] and oligimerization,
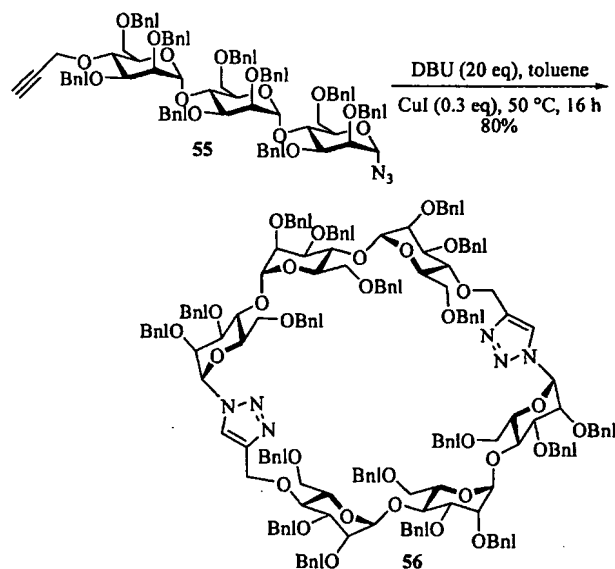
Scheme 14. Dimerization of the trisaccharide 55.

but precisely why remains unclear. Additional examples might distinguish between whether these conditions in general mediate successful intramolecular triazole formation or effects specific to the substrate dominate in determining optimal conditions.

3.4 Problematic Alkyne–Azide Cycloadditions: When Click Reactions Fail to "Click"

Overall, Cu^I-catalyzed alkyne–azide cycloaddition generates triazoles with outstanding reliability and efficiency, but as with any reaction, some problems do exist. In a few cases, various researchers have reported byproduct formation due to alkyne homocoupling, a process which copper also catalyzes.[10,36h] Further, azide binding may prove problematic for highly electron-deficient azides[47f] or for polyalkyne substrates that have the flexibility to coordinatively saturate Cu^I.[36h]

3.4.1 Alkyne Homocoupling

As the numerous reactions delineated above indicate, click chemistry nearly always proceeds in high yield with no byproduct formation. In the unusual case of a low-yielding reaction, Cu-catalyzed acetylenic homocoupling is the most likely culprit (Scheme 15).[67] Since small, unhindered amines, such as pyridine and TMEDA, mediate this conversion through stabilization of intermediates **58** and **59**,[68] low yields reported by Wong and co-workers for alkyne–azide cycloaddition under conditions of CuI/Et$_3$N (Table 4, Entry 2) likely result from increased alkyne homocoupling. Increasing the steric bulk in a base reduces its ligand donor properties, implying that sterically hindered bases should stabilize copper acetylide intermediates **58** and **59** to a lesser degree and slow this side reaction.
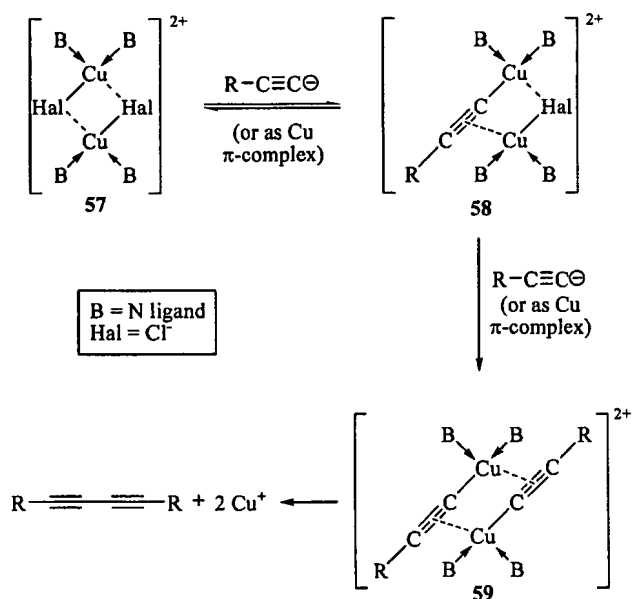
Scheme 15. Proposed mechanism for Cu-catalyzed acetylenic coupling.

3.4.2 Cu^I Saturation

As described above, Cu^I-catalyzed alkyne–azide cycloaddition requires labile ligands around the Cu^I to enable competitive azide binding. For flexible polyalkynes such as tetraalkyne **60**, the proximity of the alkynes may in effect coordinatively saturate the Cu^I atom through chelation (Scheme 16).[36h] Although Zhao and co-workers successfully synthesized triazole-functionalized calixarene **27** from calixarene azide **26** (Table 2), they were unable to effect conversion of calixarene alkyne **60**, even at elevated temperatures. Presumably, formation of a copper–acetylide species on one of the alkyne chains positioned the Cu^I ion in the vicinity of the neighboring alkyne units, which subsequently formed complexes with the Cu^I and precluded the binding of azide **61**. Complex mixtures were obtained from reactions at elevated temperatures, most likely resulting from alkyne homocoupling.[36h]
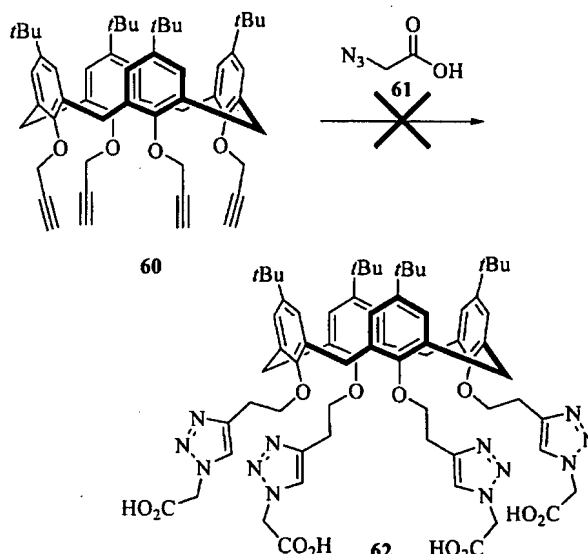
Scheme 16. Failed cycloaddition of alkyne **60** and azide **61**.

Conclusions

Considering its impressive scope, yields, and reliability, Cu^I-catalyzed alkyne–azide coupling deserves to be designated "click chemistry." Perhaps in part due to this robustness, a variety of conditions affords the desired product with comparable catalyst loading, reaction time, product purity, and yield. Factors such as solubility, the need for an inert atmosphere, or particular substrate effects may weigh more heavily on the success of a reaction than any general set of conditions. On the whole, catalyst generation through Cu^I salt addition with excess of a hindered base seems to give the best results for traditional solution-phase chemistry, but other methods clearly work well also, particularly for biological systems.

Although the scope of this reaction is indeed remarkable, not every azide or alkyne gives good results. Highly electron-deficient fluorine-substituted azides react sluggishly with low yields in general (Table 5, Entry 6), and sulfonyl-

substituted azides in the presence of Cu^I, acetylenes, and amines give N-sulfonylamines, and no triazole product,[69] indicating that alkyne–azide coupling requires a more electron-rich azide. In contrast, electron-poor alkynes actually accelerate the reaction by facilitating the formation of the metallocycle **8** (Scheme 3).[10,26] Steric constraints, particularly on the solid phase (Table 10, Entry 1b), may also limit the success of this reaction, but only in extreme cases. Otherwise, alkyne and azides for the most part "click" together, just as expected.

With such a powerful reaction, applications in combinatorial chemistry, organic synthesis, bioconjugation, and other fields are just beginning to be explored. As continued mechanistic studies reveal more about important copper intermediates, optimal conditions may be easier to predict, leading to even greater reliability and further application. Since the discovery of Cu^I-catalyzed click chemistry in 2002, interest and applications of triazoles has increased dramatically, with no sign of slowing down.

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Efficient synthesis of 1,4-disubstituted 1,2,3-triazoles in ionic liquid/water system

Ya-Bin Zhao, Ze-Yi Yan and Yong-Min Liang*

State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, PR China

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Abstract—A copper(I) catalyst in a mixture of the ionic liquid [bmim][BF$_4$] and water, can effect three-component reaction of halides, sodium azide and alkynes to form 1,4-disubstituted 1,2,3-triazoles in good to high yields. The method is efficient and environmentally friendly.
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1,2,3-Triazoles are important five-membered nitrogen heterocycles, involved in a wide range of industrial applications such as agrochemicals, corrosion inhibitions, dyes, optical brighteners and biologically active agents.[1] Earlier, the compounds are in general prepared through the coupling reaction between alkynes and azides to form a mixture of 1,4-substituted- and 1,5-substituted-1,2,3-triazoles at high temperature.[2] The recent important investigations led to the 'click' chemistry approach.[3] Sequentially, the copper(I)-catalyzed Huisgen cycloaddition reaction of azides and terminal alkynes has emerged as a novel alternative, and received much attention since its discovery. The methodology has found applications in drug discovery, bioconjugations and materials science.[4] Exclusive regioselectivity, wide substrate scope and mild reaction conditions have made it the method of choice for making permanent connections by means of 1,4-disubstituted 1,2,3-triazoles. For instance, in the last few years, the copper(I)-catalyzed Huisgen cycloaddition reaction has been widely used in multi-component reactions (MCRs) to prepare 1,2,3-triazoles with various functional groups.[5]

Room temperature ionic liquids are environmentally benign solvents due to their unique chemical and physical properties, and have proved to be especially useful in the case of catalytic reactions.[6] Ionic liquid has also shown promising results, such as hydrogenations,[7] Diels–Alder reaction,[8] enantioselective allylation reactions[9] enantioselective epoxidation of alkenes[10] and enantioselective ring opening of epoxides.[11] Although coupling reactions of aryl halides with sodium azide was reported,[12] and Zhong and Guo developed a thermal 1,3-dipolar cycloaddition of alkynes to azides in ionic liquid,[13] however, no attempt has been made for the use of ionic liquids in copper(I)-catalyzed Huisgen cycloaddition process. Herein, we reported an efficient and safe recyclable one-pot three-component reaction in ionic liquid to form 1,4-disubstituted 1,2,3-triazoles. Reaction of various halides, sodium azide with terminal alkynes in [bmim][BF$_4$]/H$_2$O afforded corresponding triazoles in good to excellent yields (Scheme 1).

Initially, we investigated the catalytic activity of various copper catalysts for the MCR of benzyl chloride,
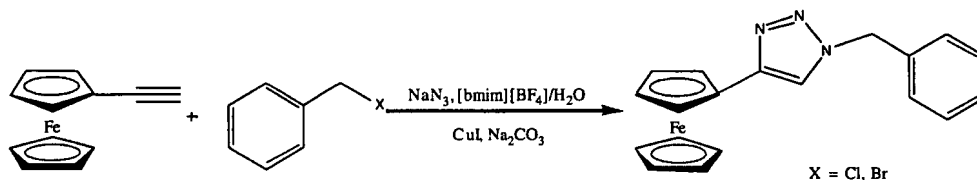
Scheme 1.

* Corresponding author. Tel.: +86 931 8912596; fax: +86 931 8912582; e-mail: liangym@lzu.edu.cn

Table 1. Evaluation of catalysts in the reaction[a]

Entry	Catalyst	Time (h)	Yield (%)[b]
1	CuCN	6	72
2	CuBr	6	65
3	CuCl	6	45
4	CuI	4	75
5	CuSO₄/Vc	8	63

[a] All reactions were carried out using 0.5 mmol benzyl chloride, 0.5 mmol phenylacetylene, 15 mol % copper salts catalyst, 0.6 mmol NaN₃ and 1 mmol Na₂CO₃ in 4 ml [bmim][BF₄] at room temperature.
[b] Isolated yield.

Table 2. Evaluation of various solvents for the reaction

Entry	Solvent	Time (h)	Yield (%)[a]
1	DMSO	4	30
2	THF	4	35
3	[bmim][BF₄]	4	75
4	[bmim][BF₄]/H₂O 1:4	4	80
5	[bmim][BF₄]/H₂O 1:1	4	94
6	DMSO/H₂O 1:4	24	90

[a] Isolated yield.

sodium azide and phenylacetylene in ionic liquid. As expected, the cycloaddition reaction proceeded well in the presence of 15 mol % of copper salts (CuI, CuBr, CuCl, CuCN and Vc-CuSO₄ system) to give triazole in 45–75% yield (Table 1, entries 1–5). But, compared to 75% yield in [bmim][BF₄], only trace of the corresponding product was isolated by using CuI in water. Sole

Table 3. Recycle of [bmim][BF₄]/H₂O reaction medium

Entry	Cycle	Time (h)	Yield (%)[a]
1	0	4	94
2	1	3.5	91
3	2	4	92
4	3	5	93
5	4	4	92

[a] Isolated yield.

copper(II) catalysts such as CuBr₂, CuCl₂ and CuSO₄ showed no catalytic effect. Although some workers reported that copper metal could catalyze the cycloaddition in low yield, in this study, no reaction occurred in the presence of copper(0) either copper powder or copper turning.[14] Attempts to explore other metal catalysts such as AgCl, AgBr, AgI and ZnCl₂ failed. Although all Cu(I) species showed the good catalytic properties, the best effect was observed by using CuI (Table 1, entry 4).

We also evaluated the solvent effect in the three-component reaction. The results evaluating the merits of various solvents are presented in Table 2. It was shown that ionic liquid was a type of feasible reaction medium in Huisgen cycloaddition. In comparison with the reactions in organic solvent and water, it only took 4 h in ionic liquids to give moderate yield. To our surprise, using [bmim][BF₄]/H₂O 1:1 instead of [bmim][BF₄], the corresponding product was obtained in excellent yield (Table 2, entry 5). We presumed that water played a positive role in the present system due to good water solubility of sodium azide.[15]

Table 4. Reactions of halides, alkynes and NaN₃ to form corresponding triazoles
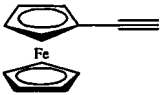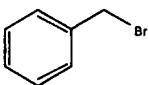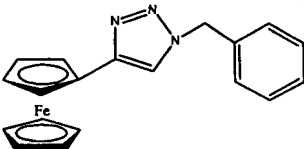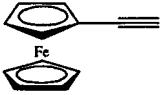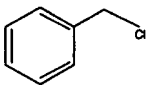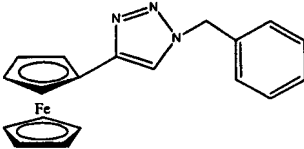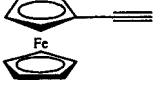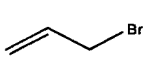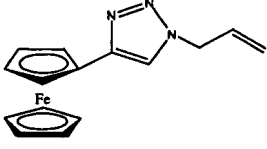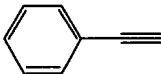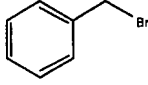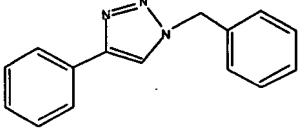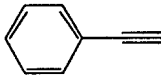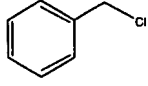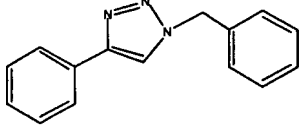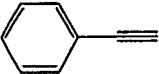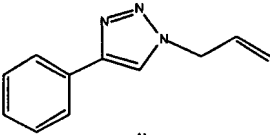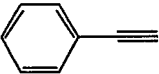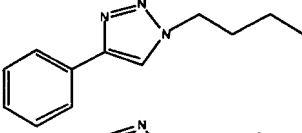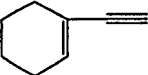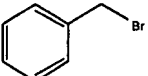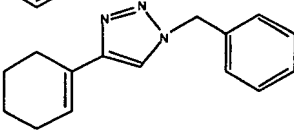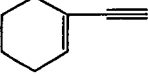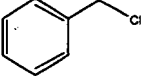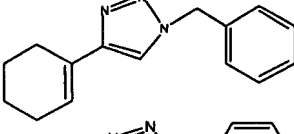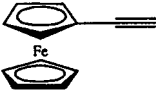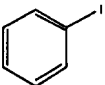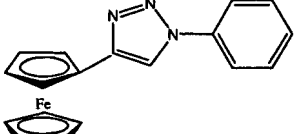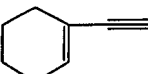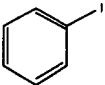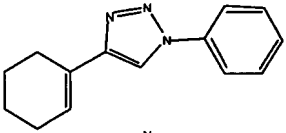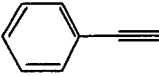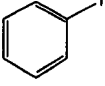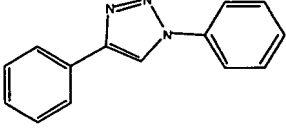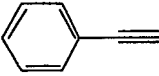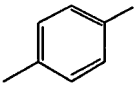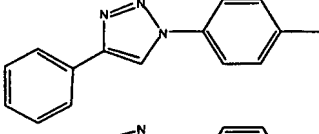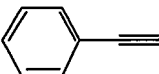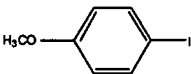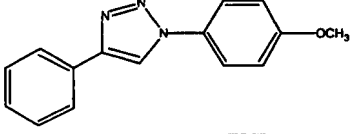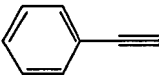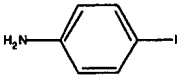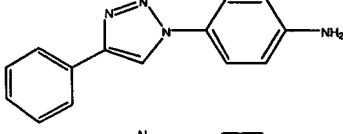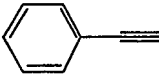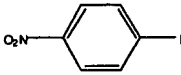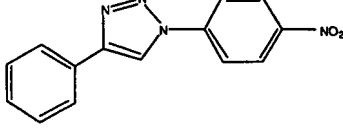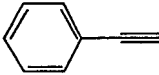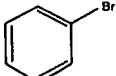
Entry	Alkyne	Halide	T (°C)	Time (h)	Product	Yield (%)
1			rt	8		99
2			rt	8		97
3			rt	10		75
4			rt	4		97
5			rt	4		94

Table 4 (*continued*)

Entry	Alkyne	Halide	T (°C)	Time (h)	Product	Yield (%)
6			rt	5		76
7			rt	5		90
8			rt	4		96
9			rt	4		95
10			65	8		80
11			65	7		72
12			65	6		83
13			65	8		70
14			65	8		75
15			75	10		72
16			80	12		68
17			100	20	No reaction	

Having optimized the reaction conditions, the recycl-ability of three-component reaction with copper(I) catalysts in [bmim][BF₄]/H₂O was examined (Table 3).

Similar high conversions were obtained, showing that the ionic liquid remained active. Furthermore, no significant loss of the ionic liquid was observed after five
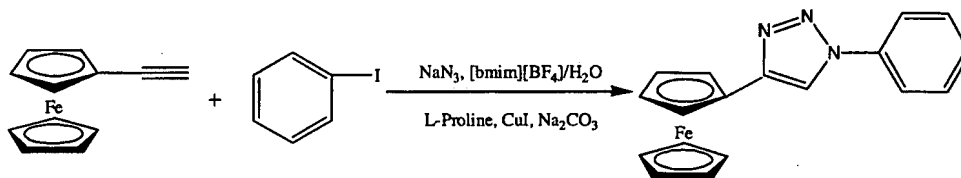
Scheme 2.

cycles, which indicated that it could be recycled efficiently in this way.

Having established the recyclability and reuse of the ionic liquid, the scope and generality of this method was illustrated with respect to various triazoles (Table 4). It is important to stress that we explored the Huisgen reaction of ferrocenylacetylene because ferrocene-containing heterocyclic rings were reported to be useful materials in electrochemistry, biochemistry and material science.[16] Up to now, although the Cu(I) catalyzed reaction of alkynes and azides have been widely reported, no attempt was carried out to synthesize triazoles including ferrocene segment. Herein, we were pleased to find that ferrocenylacetylene would be compatible with azide and copper catalyst.[17]

In order to understand more about the effect of the copper(I)-catalyzed Huisgen cycloaddition reaction in ionic liquids, we extended our study by using halides at sp^2-hybridized carbon atoms (phenyl halides). At room temperature, it was failed to complete the reaction of phenyl halides. Thus, we had to explore new reaction system (Scheme 2). The reaction temperature and various auxiliary conditions were examined.[18] We found that the reaction of iodobenzene, alkynes and sodium azide proceeded well in the presence of 20% L-proline at 65 °C, and gave the corresponding products in 68–83% conversion (Table 4, entries 10–16). An attempt to extend the reaction to bromobenzene was proved to be not effective. No expected product was isolated even elevating the temperature to 100 °C.

In conclusion, we found that the ionic liquid/H_2O was a good reaction medium for the one-pot synthesis of 1,4-disubstituted 1,2,3-triazoles using either halides at sp^3-hybridized carbon atoms or halides at sp^2-hybridized carbon atoms in good yields. Better performance was obtained than that of conventional reaction condition. This procedure permits extensive recycling of the solvent without substantial loss in activity within five times. On the basis of the current efforts, it seems to be likely that a number of metal catalyzed transformations could be performed equally well or even more effectively in this ionic liquid. Studies to determine applicability to other catalytic reactions are currently underway in our lab.

Acknowledgements

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17. Representative procedure: benzyl chloride (64 mg, 0.5 mmol), phenylacetylene (52 mg, 0.5 mmol) and NaN$_3$ (39 mg, 0.6 mmol) were placed in a 10 ml round-bottomed flask. Sequentially, Na$_2$CO$_3$ (106 mg, 1 mmol) and CuI (15 mol %) were added, the reaction mixture was stirred in 4 ml [bmim][BF$_4$]/H$_2$O 1:1 at room temperature for 4 h and then extracted with ether (3 × 10 ml). The combined organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$ filtered and concentrated in vacuo. The residual crude product was purified via silica gel. Ionic liquid was recovered by extracting with dichloromethane and can be reused for the same reactions. 1-Benzyl-4-cyclohex-1-ene-1-*H*-[1,2,3]triazole: ^{1}H NMR (CDCl$_3$, 300 MHz, δ ppm): 7.32 (s, 1H), 7.30–7.17 (m, 5H), 6.43 (t, H), 5.41 (s, 2H) 2.29 (t, 2H), 2.11 (t, 2H), 1.56–1.71 (m, 4H); ^{13}C NMR (CDCl$_3$, 75 MHz, δ ppm): 150.3, 135.5, 129.4, 128.9, 128.3, 127.8, 125.3, 118.9, 118.8, 54.3, 26.8, 25.7, 22.9, 22.7; MS: $m/z = 239$ (M$^+$), 210, 182, 120, 91, 77. IR (KBr, cm^{-1}): 3103, 2927, 1454, 1431, 1215, 1070.
18. Procedure for the reaction of phenyl iodide: other reaction conditions are the same as benzyl chloride, reaction could proceed in the presence of L-proline (24 mg, 20 mol %) at 65 °C. 1-(4-Methylphenyl)-4-phenyl-1-*H*-[1,2,3]triazole: ^{1}H NMR (CDCl$_3$, 300 MHz, δ ppm): 8.16 (s, 1H), 7.91 (d, $J = 8.1$, 2H), 7.60 (d, $J = 8.1$, 2H), 7.48–7.45 (m, 2H), 7.39–7.31 (m, 3H), 2.43 (s, 3H); ^{13}C NMR (CDCl$_3$, 75 MHz, δ ppm): 148.2, 138.8, 134.7, 130.3, 130.2, 128.9, 128.3, 125.8, 120.4, 117.6, 21.1; MS: $m/z = 235$ (M$^+$), 206, 116, 91. IR (KBr, cm^{-1}): 3124, 1520, 1230, 1044.

Inhibitors of HIV-1 Protease by Using In Situ Click Chemistry**

*Matthew Whiting, John Muldoon, Ying-Chuan Lin,
Steven M. Silverman, William Lindstrom,
Arthur J. Olson, Hartmuth C. Kolb, M. G. Finn,
K. Barry Sharpless, John H. Elder,* and
Valery V. Fokin**

The global AIDS epidemic has claimed the lives of more than 20 million people since 1981. Another 10 million are now living with HIV and most of these are likely to develop AIDS over the course of the next decade. In spite of the various treatment protocols available, including the mainstream

[*] Dr. Y.-C. Lin, Dr. W. Lindstrom, Prof. A. J. Olson, Prof. J. H. Elder
Department of Molecular Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-2750
E-mail: jelder@scripps.edu

Dr. M. Whiting, Dr. J. Muldoon, S. M. Silverman, Prof. H. C. Kolb,
Prof. M. G. Finn, Prof. K. B. Sharpless, Prof. V. V. Fokin
Department of Chemistry and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road, BCC-315, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-7562
E-mail: fokin@scripps.edu

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highly active antiretroviral therapy (HAART),[1] the number of people infected with HIV continues to rise. The most recent UNAIDS (United Nations Programme on HIV/AIDS)/world health organization estimates show that, in 2004 alone, 4.9 million people were newly infected with HIV.[2]

HIV-1 protease (HIV-1-Pr)[3] has been recognized as an important target for inhibition of viral replication. Although seven inhibitors have been approved by the food and drug association since 1995 and a number more are currently undergoing clinical evaluation, their success has been undermined by rapid mutation of the virus.[4] The alarming rate at which strains of HIV-1 that are resistant to the currently available drugs and their combinations emerge underscores the urgent need for new, broad-spectrum protease inhibitors that are effective against the new mutants as well as the wild-type virus.

Herein we describe the early results of our investigation into the application of a multidisciplinary, chemistry-driven approach for the rapid discovery of bioactive molecules, termed in situ click chemistry, for the elucidation of novel HIV-1-Pr inhibitors. The goal of in situ click chemistry is to accelerate the identification of novel pharmaceutical candidates through involvement of a biological target in the selection and covalent assembly of its own inhibitors. Although the concept has been previously demonstrated by several researchers,[5] the in situ click-chemistry approach[6] is unique in that it relies on the completely bio-orthogonal 1,3-dipolar cycloaddition of organic azides and alkynes.[7] This highly exergonic reaction produces five-membered nitrogen heterocycles, 1,2,3-triazoles, which are exceedingly stable to acidic and basic hydrolysis as well as severe reductive/oxidative conditions. At the same time, the triazoles produced are capable of active participation in hydrogen bonding as well as dipole–dipole and π-stacking interactions.

Even though both azides and alkynes are energetic species, their reactivity profiles are quite narrow, at least under physiological conditions. Furthermore, despite the large thermodynamic driving force for cycloaddition, the high kinetic barrier effectively hides the reactants until they are brought into close proximity by a biological template or activated towards each other by alternative means (e.g., catalysis). These features allow the target to sample numerous combinations of building blocks, but synthesize only the best binders. The efficacy of in situ click chemistry has already been demonstrated by the discovery of novel, highly potent inhibitors of acetylcholinesterase and carbonic anhydrase.[6] Previous studies have, however, always utilized building blocks with a high affinity for the target ($K_i < 37$ nM).[6c,d] In the HIV-1-Pr case, we do not have such a luxury and as such,

we were pleased to find that the enzyme selectively formed an inhibitor from components that exhibited only weak binding to the target.

To probe the protease-templated reaction, alkyne **1** (500 μM, $IC_{50} > 100$ μM) and azide **2** (100 μM, $IC_{50} = 4.2 \pm 0.6$ μM) were incubated in the presence of the HIV-1,-Pr SF-2-WTQ7K-Pr,[8] (henceforth simply denoted SF-2-Pr; 15 μM) in 2-morpholinomethanesulfonic acid (MES; 0.1M)/NaCl (0.2M) buffer solution at 23°C for 24 h (Scheme 1). Controls
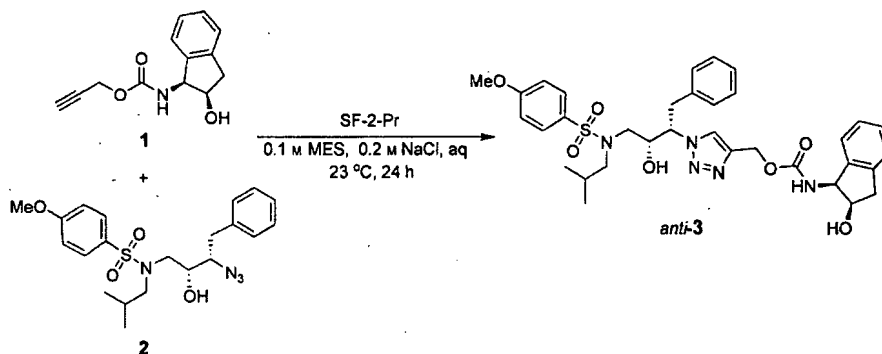
Scheme 1. SF-2-Pr templated in situ click-chemistry formation of protease inhibitor *anti*-3.
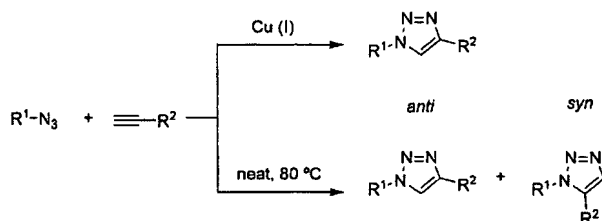
were performed both in the absence of the enzyme, to confirm the background rate of the cycloaddition, and in the presence of bovine serum albumin (BSA), to determine any nonspecific peptidic catalysis. Analysis of the crude reaction mixtures was carried out by HPLC and mass spectrometry in single-ion mode (LCMS-SIM), which was previously demonstrated to be a sensitive, medium-throughput method for the detection of a desired product.[6b] Comparison of the enzyme and background reactions showed that the triazole *anti*-3, which has previously been shown to be an inhibitor of the wild-type HIV-1-Pr ($IC_{50} = 6$ nM, $K_i = 1.7$ nM) and also of several mutant strains,[9] was indeed formed in an increased rate in the presence of the enzyme. In contrast to other systems,[6b,c] the background reaction was observable under our chosen conditions and produced a detectable amount of the regioisomeric triazoles *anti*- and *syn*-3 in a 2:1 ratio, favoring the 1,4-disubstituted product *anti*-3. Surprisingly, the presence of BSA gave an increase in the observed rate of product formation, which led to a small increase in the final product concentration without affecting the regioisomeric ratio. However, a marked acceleration in the rate of formation of only one of the regioisomers was observed in the presence of the enzyme with an approximately tenfold increase in the overall product formation. The triazole product was formed with a much enhanced regioisomeric ratio of 18:1 in favor of the same 1,4-isomer, *anti*-3 (Figure 1). In a further control experiment, a known HIV-1-Pr active-site ligand, TL3[10] (15 μM), was added to both the background and enzyme reactions prior to incubation with the azide and alkyne fragments. The added inhibitor showed no effect on the background reaction[11] but completely suppressed the increase in product formation in the presence of the enzyme,
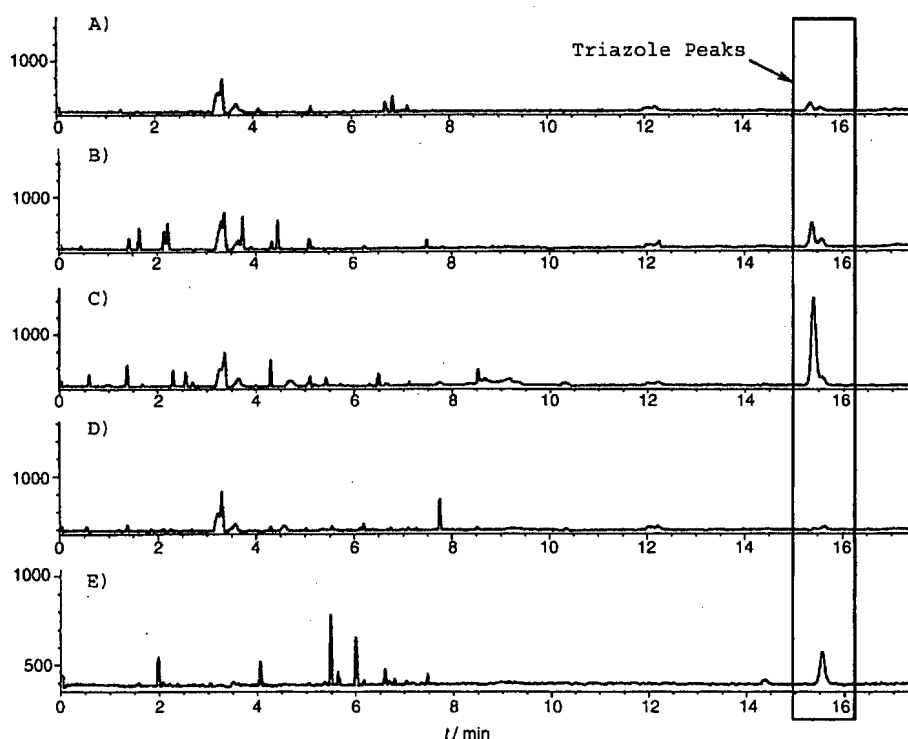
Figure 1. In situ formation of **3** determined by LCMS-SIM. A) Background reaction, alkyne **1** (500 μM) and azide **2** (100 μM) were incubated in MES (0.1 M) and NaCl aqueous buffer (0.2 M) for 24 h. B) Compounds **1** and **2** incubated in the presence of BSA (0.5 mg mL⁻¹, 7.5 μM). C) Compounds **1** and **2** were incubated in the presence of SF-2-Pr (15 μM). D) Compounds **1** and **2** incubated in the presence of SF-2-Pr (15 μM) and protease inhibitor TL3 (15 μM). E) Authentic sample of *anti*-**3**.

We next investigated whether the enzyme could select a pair of fragments that form a potent binder from a number of building blocks that are concurrently present in a single reaction mixture. In the context of a drug-discovery program, this would be a major advantage, enabling more-rapid screening and reducing the amount of enzyme required for each individual analysis. In the present system, azide **2** was known to be the tightest-binding fragment; we therefore hoped to show that it could recruit a complimentary fragment from an array of alkynes. Alkynes **4–7** (Figure 2) were chosen as test substrates owing to their similar molecular weight and degree of functionality when compared with **1**. It was also determined that, as expected, none of the individual fragments gave strong protease inhibition (IC₅₀ of Alkynes **4–7** > 100 μM).
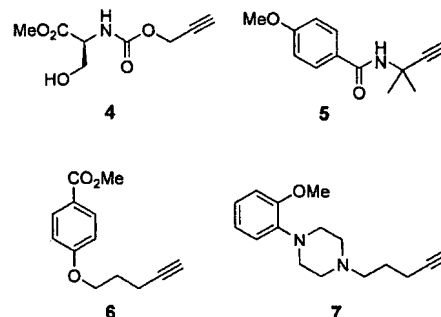
thereby demonstrating that the active site does indeed act as a template for the cycloaddition reaction. The reaction, performed in situ, was also carried out in the presence of the more clinically relevant HIV-1-Pr, NL4-3-WTQ7K-Pr,[12] which gave essentially identical results.

To determine the regiochemistry of the triazole formed by the enzyme, a mixture of both regioisomers, *syn*- and *anti*-**3**, was obtained by thermal cycloaddition, and the regioisomerically pure 1,4-disubstituted triazole product, *anti*-**3**, was prepared by a copper(I)-catalyzed reaction of the corresponding azide and alkyne blocks[13] (Scheme 2). Co-injection of the crude, enzyme-promoted reaction with both a mixture of the triazoles and the pure 1,4-regioisomer revealed that the enzyme had preferentially formed the expected 1,4-triazole, *anti*-**3**.[11]
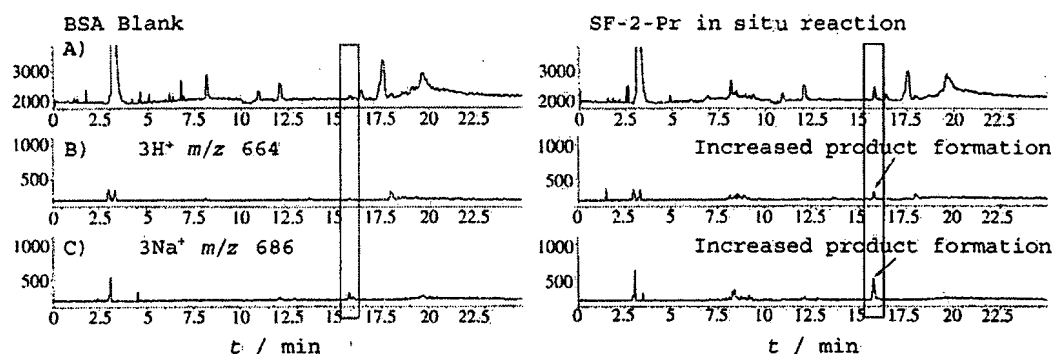
Thus, instead of screening binary mixtures of azide and alkyne fragments, azide **2** (100 μM) was incubated with the five alkynes **1** and **4–7** (200 μM each) in the presence of both SF-2-Pr (15 μM) and BSA (to monitor the background reaction). After incubation for 24 h at 23°C, the crude reaction mixtures were directly analyzed by LCMS-SIM (monitoring for MH⁺ and MNa⁺ of all potential products[14]). Comparison of the total ion count and the extracted-ion traces of each possible product from the enzyme and background reaction showed an increased amount of only one triazole product (**3**) in the presence of the enzyme (Figure 3).[15]

Scheme 2. Preparation of regioisomeric triazoles by copper(I)-catalyzed and thermal cycloaddition reactions.

Figure 2. Alkynes **4–7**.

Figure 3. LCMS-SIM and extracted-ion traces for the in situ mixture experiment as described above. The combined traces (A) are shown as well as the extracted-ion traces for both $3H^+$ (B) and $3Na^+$ (C). The left-hand traces show the BSA background reaction and the right-hand traces show the corresponding in situ reaction with SF-2-Pr. Extracted-ion traces for all alternative-product masses showed no increased product formation.[11]

In summary, this work has demonstrated that the in situ click-chemistry approach can be successfully applied to the formation of HIV-1-Pr inhibitors. The protease itself acts as a template for the reaction and greatly increases the rate of formation of the 1,4-triazole product, *anti*-3. This rate acceleration is readily detected by simple comparison with the LCMS-SIM traces for the in situ reaction and a background reaction by using BSA in place of the enzyme. In this study, the use of building blocks with much lower affinities for the biological target than in previous examples should greatly facilitate the transfer of this technology to new systems in which the structures of high affinity binders are not known. Further studies are currently underway that apply this methodology to the discovery of entirely novel protease inhibitors by using even lower affinity fragments as well as efforts to directly employ drug resistant, mutant proteases in the in situ discovery process.

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[14] There is a possibility that potential products could be mass-spectrometry silent and thus would be left undetected if they had significantly different ionization energies. In the present study this was demonstrated not to be the case as the common azide fragment 2 gave a relatively large ESI-MS signal compared with alkyne 1 (see the Supporting Information for a more detailed explanation).

[15] One advantage of the in situ click-chemistry approach over the traditional drug-discovery techniques is that the need to independently synthesize each individual compound for screening is eliminated. Increased rate of formation of a triazole

product(s) indicates possible hits that can then be further investigated individually (again, very quickly thanks to the "guaranteed" nature of the cycloaddition reaction). To ascertain that no binders were overlooked in this particular case, we also carried out thermal cycloaddition reactions of each individual azide/alkyne combination and determined the product retention times by LCMS. This confirmed our initial determination that only one triazole was formed with an increased rate in the presence of the biological target, thereby illustrating that the enzyme was able to sample an array of potential inhibitor fragments and select only one productive combination, the one leading to the inhibitor 3.

The potential of cycloaddition reactions in the synthesis of dendritic polymers†

Brigitte Voit

Cycloaddition (CA) reactions have attracted recently strong interest not only for the preparation of linear polymers but also for the synthesis and modification of dendritic architectures. This review focuses mainly on the potential of various cycloaddition reactions in the field of dendrimers and especially hyperbranched polymers. The [4 + 2] Diels–Alder cycloaddition, [2 + 2 + 2] CA as well as 1,3-dipolar CA reactions including "click chemistry" will be addressed, and advantages of these reactions will be highlighted. High selectivity, thus high tolerance towards additional functionalities, high yields and often moderate to mild reaction conditions distinguish CA reactions from the often applied classical high-temperature polycondensation type reactions used mainly to synthesize hyperbranched polymers. Thus, besides the high potential in the synthesis and modification of perfectly branched dendrimers, cycloaddition reactions proved also very suited to prepare new types of hyperbranched structures.

Introduction

Dendritic polymers, the perfectly branched dendrimers as well as their hyperbranched cousins and a variety of combinations between dendritic and linear structures, are certainly among the most studied functional and specialty polymers today. Due to the highly branched, compact and highly functional structure with special reactivity, solubility and rheology features,

Leibniz-Institut für Polymerforschung Dresden, Hohe Strasse 6, 01069 Dresden, Germany. E-mail: voit@ipfdd.de
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Brigitte Voit received her PhD in Macromolecular Chemistry in 1990 from University Bayreuth, Germany. After postdoctoral work at Eastman Kodak in Rochester, USA, she went to Technische Universität München in 1992, receiving her habilitation degree in Macromolecular Chemistry there in 1996. In 1997, Brigitte Voit was appointed head of the Institute of Macromolecular Chemistry at the Leibniz Institute of Polymer Research (IPF) Dresden, as well as professor for "Organic Chemistry of Polymers" at the University of Technology Dresden. Since 2002, she is heading the IPF Dresden as the Scientific Director. Her major research topics cover hyperbranched (dendritic) polymers, functional polymers, block and graft copolymers, as well as thermo- and photo labile polymers with strong synthetic aspects.

applications in nanotechnology, biomedicine, sensors, and micro(nano)electronics, but also in a variety of reactive formulations are discussed. The reactions explored so far for the synthesis of dendritic polymers are numerous and are covered in a number of excellent reviews.[1–11] Nevertheless, to achieve high control of the structure and high yields in the polyreactions are still major issues which need to be addressed, and the lack of these are considered together with the restricted monomer availability and the high costs the limiting factors for the market introduction of dendritic polymers.

Cycloaddition (CA) reactions are known to exhibit the features needed urgently in dendrimer synthesis: they are very efficient and highly selective and allow even stereoselectivity! CA reactions have been used successfully in polymer synthesis already for 40 years,[12] an area reviewed excellently very recently by Goodall and Hayes[13] who also pointed out already the developments in dendrimer synthesis.

Therefore, one is not surprised that besides Michael *addition* reactions, which are used very successfully for the synthesis of polyamidoamine (PAMAM) and polypropyleneimine dendrimers,[1,2] special types of *cycloaddition* reactions have been also early on explored in the preparation of dendrimers.[14–16] Moving from addition to cycloaddition reactions offers even more control over the polyreaction with the potential to form fully new structural units and to achieve also stereo-control and even unusual effects regarding the degree of branching (DB) in hyperbranched products through the formation of special intermediate structures during the growth of the macromolecules. Even in the classical Michael addition reaction positive effects on the DB were observed. Hobson and Feast[17] reported poly(amido amine)s (PAMAM like) hyperbranched polymers using an amino-acrylamide AB₂ monomer. Unexpectedly, a degree of branching close to 100% was determined which could not be fully explained but it can be

assumed that a special intermediate is formed during the reaction favoring the second branching.

However, only recently, especially with further developments concerning the Huisgen 1,3-dipolar cycloaddition[18,19] towards the so-called "click chemistry",[20,21] the use of CA reactions has grown nearly exponentially, for both the preparation and functionalization of dendrimers and dendrimer–linear hybrids. In addition, CA reactions are also used very successfully in the preparation of hyperbranched polymers.

This perspective will thus point out these recent developments concerning the use of CA reactions for the synthesis and modification of dendrimers and especially hyperbranched polymers.

Cycloaddition reactions

Cycloaddition reactions[22] lead to the formation of new σ bonds via cyclic transition states which finally results in a cyclic product. In the intermediate state phase matched interactions between the highest occupied molecular orbital (HOMO) of one component and the lowest unoccupied molecular orbital (LUMO) of the other compound are involved which results in the formation of bonds in a concerted and thus highly controlled manner.[23] CA reactions are usually affected only to a very small extent by the medium used and they can be promoted by temperature or by light.[24]

The subclasses of these reactions are classified by the number of π electrons that are involved in the CA reaction e.g. [4 + 2] or [2 + 2]. The 1,3-dipolar CA also fits in this classification: it involves 6 π electrons and is denoted as [4π + 2π] (or [4 + 2]) type cycloaddition. However, it is often described as a (3 + 2) process to distinguish it from other [4 + 2] CAs which can cause some misunderstanding. Details on the mechanism and selection rules of the different CA reactions can not be given here and thus one has to refer to the literature.[18,19,25] However, it may be noted, that orbital symmetry controlled CA reactions will be antarafacial and suprafacial, according to the Woodward and Hoffmann selection rules, and this depends on the number of electrons involved as well as on the type of promotion (thermal or photochemical). In addition, other cycloadditions may occur which involve
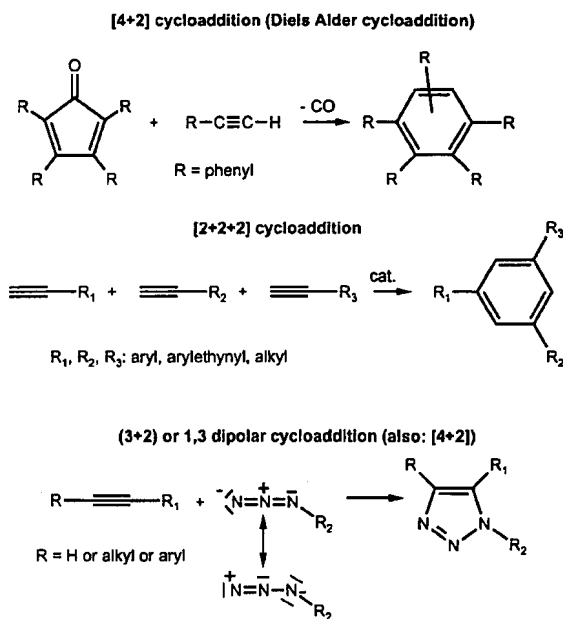

Scheme 1 Examples for some cycloaddition reactions employed for the synthesis of dendritic macromolecules.

intermediate steps and which do not necessarily follow the Woodward–Hoffmann selection rules.

In the synthesis of dendritic polymers so far mostly the [2 + 2 + 2] (reaction of three alkynes), the [4 + 2] CA (Diels–Alder: cyclopentadione + alkyne or maleimide) and the 1,3-dipolar CA (alkyne + azide or bisazine + maleimide) have been used. Scheme 1 exemplifies the mainly used reactions schematically.

Cycloaddition reactions and polyarylenes

Polyphenylenes are an interesting class of materials due to a usually high thermostability and chemical inertness. Furthermore, the highly aromatic nature renders these structures suitable for a number of technological applications e.g. in microelectronics implying also the potential to include controllable conductive behavior. Early on, CA reactions were
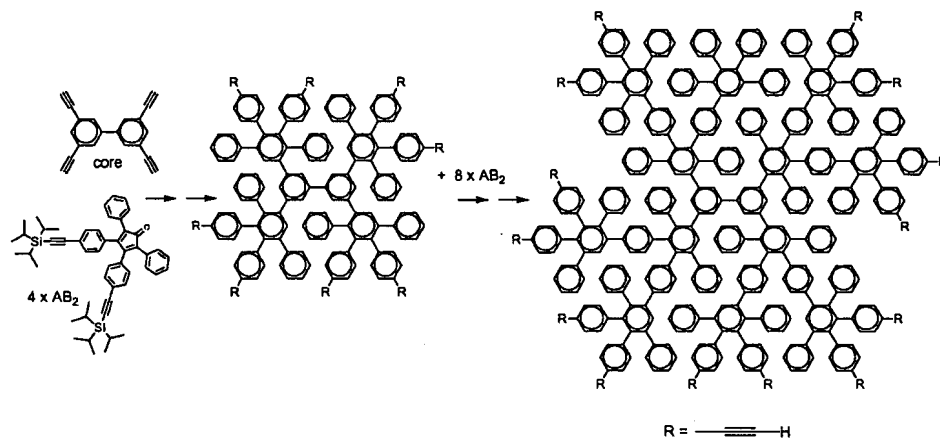
Scheme 2 Divergent synthesis of polyphenylene dendrimers through Diels–Alder reaction adapted from Müllen and co-workers.[15]
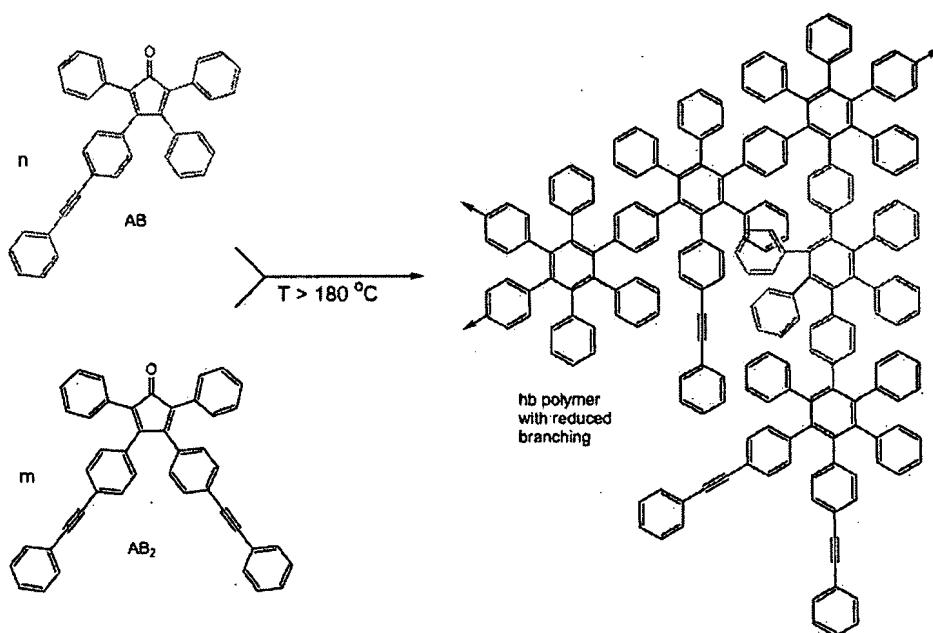
Scheme 3 Synthesis of hyperbranched polyphenylenes with an increased fraction of linear units by Diels–Alder reaction from AB₂ and AB monomers (adapted from ref. 50).

employed also for the preparation of linear polyphenylenes, exemplified mostly by work reported by Stille et al.[26,27]

Introducing branched units allows to overcome a major drawback of these structures, which is the limited processibility. Already in 1992 Miller et al.[28] reported the synthesis of perfectly branched polyphenylene dendrimers and their fluorinated analogues by a palladium-catalyzed coupling of arylboronic acids to 3,5-dibromo-1-(trimethylsilyl)benzene. But polyphenylene dendrimers based on the Diels–Alder reaction had been the issue of one of the first reports on the use of CA reactions in dendrimer synthesis.[14–16] This synthetic approach is based on the cycloaddition of phenylated cyclopentadienones with phenylated alkynes and subsequent decarbonylation (see Scheme 1). In an early attempt, Wiesler and Müllen[29] have used a convergent approach using 2,3,4,5-tetraphenyl-cyclopenta-2,4-dienone with 4,4′-diethynylbenzil to form the first-generation dendritic precursor which was further reacted with 1,3-diphenylacetone leading to the first generation dendron. However, this convergent approach was limited to the second generation, and thus, a divergent approach was employed for higher generation dendrimers as outlined in Scheme 2.[15] The AB₂ compound 3,4-bis(4-(triisopropylsilyl-ethynyl)phenyl)-2,5-diphenylcyclopentadienone was added to a multifunctional alkyne core molecule in the corresponding amount. After deprotection of the alkyne units in the resulting first-generation dendrimer by treatment with e.g. ammonium fluoride and catalytic amounts of tetrabutylammonium fluoride in THF, the reaction steps are repeated to yield the second generation dendrimer and so on.

By now a vast number of polyphenylene dendrimers with a dense packing or a more extended arrangement of benzene rings, but also including additional extended or asymmetric structural units and a variety of functional surface groups had already been prepared and characterized.[15,16,30–38]

Three-dimensional polyphenylene dendrimers are shape-persistent,[36] spherical molecules, whose structural properties allow spatial definition and topological isolation of functional groups in the center, in the dendritic scaffold, and at the periphery which renders them very interesting materials even in areas such as gene transfer or diagnostics. For examples, desymmetrized polyphenylene dendrimers[37] were prepared by the same [4 + 2] cycloaddition reaction employing monofunctionalized cyclopentadienones with polar groups as core moieties. By this, first it was possible to identify the different regioisomers which are a result of the mechanism of the Diels–Alder reaction, and secondly, these structures can be considered as the base for preparing new nanoamphiphiles. In addition, further oxidative cyclohydrogenation of the discussed polyphenylenes allows the construction of polycyclic hydrocarbon architectures,[14,16] and also the preparation of graphite-analogue thin layers and carbon nanotubes has been reported.[38,39] Dendritic polyphenylenes, modified with suitable end functionalities, have been already successfully employed in sensorics, e.g. in gold nanoparticle composite layers used in chemoresistor sensors.[40–42]

Soon not only the perfectly branched dendrimers, but also hyperbranched polyphenylenes had been explored. In general, polyphenylenes possess a high thermal and chemical stability. However, in contrast to their linear analogues, the hyperbranched polyphenylenes are non-conducting polymers, because an extended π-conjugation is hindered due to their tightly packed and strongly twisted phenylene units.[16] They show good solubility and processibility comparable to those of the corresponding dendrimers[43] but are more easily available also in larger quantities. Due to the outlined properties hyperbranched polyphenylenes are promising candidates for the application as insulating materials in microelectronics, where features like high thermal stability, good processibility
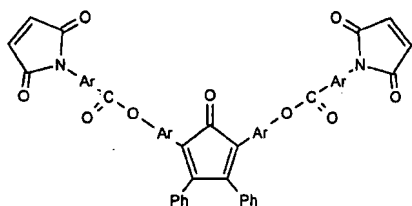
Scheme 4 AB$_2$ monomer employed by Harrison and Feast[52] for the preparation of hyperbranched poly(arylimide ester)s via a [4 + 2] cycloaddition reaction.

and solubility and low moisture absorption are required. An example for a low-dielectric-constant polymer based on branched and crosslinked polyarylenes which is already used by the semiconductor industry is SiLK™ from The Dow Chemical Company.[44,45]
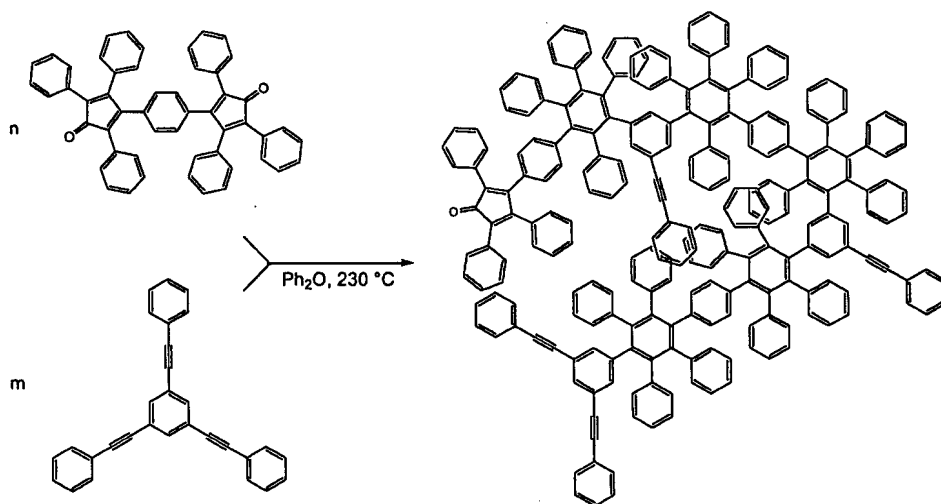
The first hyperbranched polyphenylenes were synthesized by Kim and Webster starting from (3,5-dibromophenyl)boronic acid and dihalophenyl Grignard reagents via Pd(0) and Ni(II)-catalyzed aryl–aryl coupling reactions, respectively.[46] Müllen and co-workers,[47–49] however, reported the Diels–Alder reaction of the AB$_2$ monomer 3,4-bis(4-(triisopropylsilylethynyl)phenyl)-2,5-diphenylcyclopentadienone (Scheme 2) or 3,4-bis(4-(phenylethynyl)phenyl)-2,5-diphenylcyclopentadienone (see Scheme 3). Depending on the substitution on the ethynyl group, thermal treatment at 180 °C with or without the presence of tetrabutylammonium fluoride for up to 45 h is necessary to afford the hyperbranched products with molar masses $\bar{M}_w$ up to 100 000 g mol^{-1} and broad polydispersity.[47]

This approach has been extended recently by combining the also the AB$_2$ monomer 3,4-bis(4-(phenylethynyl)phenyl)-2,5-diphenylcyclopentadienone with an AB monomer, the reaction was carried out in diphenyl ether at 230 °C (Scheme 3). Besides giving some control over the degree of branching in the branched polyphenylenes,[50] the addition of the AB monomer e.g. in a 3 : 1 (AB$_2$: AB) ratio limited the achievable molar masses to some extent: $\bar{M}_w$ values of up to 75 000 g mol^{-1} were obtained compared to up to 600 000 g mol^{-1} for pure AB$_2$

monomer used under identical reaction conditions.[50] Unfortunately, even though all the repeating units—linear, dendritic and terminal—could be identified and proven, a quantification e.g. by ^{13}C NMR analysis is not possible due to overlapping signals, and thus, no quantitative information on the degree of branching can be given. The achievement of high molar masses with relatively broad molar mass distributions ($\bar{M}_w/\bar{M}_n$ between 3 and 30) and rather multimodal GPC traces combined with an excellent solubility e.g. in chloroform or toluene are further indications for a highly branched nature of the products.[50] In addition, solid state[36] and solution[51] NMR studies proved hindered dynamics in the dendritic polyphenylenes as a result of the dense packing of the phenylene rings.

Harrison and Feast[52] reported the preparation of highly soluble hyperbranched polyarylimides using also the Diels–Alder cycloaddition reaction but employing AB$_2$ monomers with maleimide and cyclopentadienone moieties. They also react upon heating in a suitable solvent like nitrobenzene in a [4 + 2] cycloaddition reaction forming a cyclohexadiene dicarboxylic acid imide. which might be further oxidized to a benzimide unit. Unfortunately, no further characterization details of the products are given. Scheme 4 features one of the used AB$_2$ monomers, the diester monomer, similarly, a diamide monomer was successfully applied. The resulting polymers from the diester monomer were soluble in chloroform, acetone and THF whereas those from the diamide AB$_2$ compound showed limited solubility at higher molar masses probably due to strong hydrogen bonding.

There are two ways to prepare hyperbranched polymers: the classical "Flory" approach using AB$_x$ type monomers as a non-gelating system, and the more simple A$_2$ + B$_3$ approach which allows the use of more easily available monomers but implies the possibility of gelation which is only avoided by restriction of the conversion or high imbalance of the stoichiometry. Nevertheless, also by the A$_2$ + B$_3$ approach fully soluble hyperbranched polyphenylenes via the Diels–Alder reaction have been prepared[50] following the route demonstrated extensively by Müllen and co-workers.[16] Scheme 5 indicates the monomers used. It is interesting to note that by

Scheme 5 Synthesis of hyperbranched polyphenylenes by the A$_2$ + B$_3$ approach (Diels–Alder reaction).
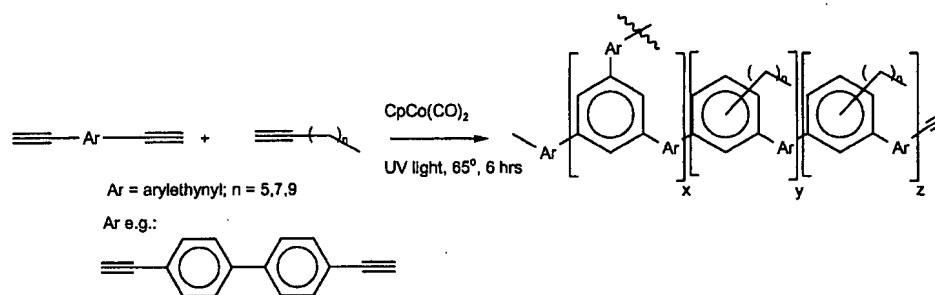
Scheme 6 Synthesis of hyperbranched polyarylethynyls by [2 + 2 + 2] cobalt catalyzed phototrimerization (adapted from refs. 13 and 54).

this monomer combination a higher steric hindrance was achieved in the final polymer structures as compared to the hyperbranched polyphenylenes prepared by the AB$_2$ monomers. Thus, detailed NMR investigations[51] revealed that the formation of linear units is favored since the reactivity of the third B unit in B$_3$ after two B's have already reacted is reduced. Only when high excess of A$_2$ is applied the formation of dendritic units becomes more significant, however, then the achievable molar mass is limited. An advantage of this fact is that also the critical conversion is shifted by this reduced reactivity and thus, only fully soluble products were obtained under the applied conditions.

Hyperbranched polyphenylenes and -arylenes are also prepared by [2 + 2 + 2] cycloaddition reactions as reported by Tang and co-workers.[53–57] Here, the diyne polycyclotrimerization initiated by transition-metal catalysts[53–56] (Scheme 6) and base-catalyzed alkyne polycyclization is applied.[56,57] In the first case, a variety of bifunctional arylene ethynylene and monofunctional alkyne monomers were combined and tantalum, niobium or cobalt catalysts were used. An optimization of the monomer combination was necessary before the system shown in Scheme 6,[54] with $n = 5$ in the alkyne monomer and under irradiated cobalt catalysis, resulted in highly soluble highly branched materials with $\bar{M}_w = 20\,000$ g mol^{-1} and an extremely high thermal stability ($T_{decomp} > 580$ °C). In this approach, the addition of the mono-alkyne avoids gelation and induces flexibility into the system leading finally to soluble products. Due to the UV irradiated cobalt catalysis, the polymerization results in a random mixture of 1,2,4- and 1,3,5-trisubsituted benzenes which makes the hb structure very irregular. Thus, the final structural features of the highly branched products are very complex. The resulting materials, however, exhibit photoluminescence and optical limiting properties and are of interest for the preparation of novel light emitting devices.

Furthermore it could be shown that the polycyclotrimerization of bis(aryl ethynyl ketones) can also be initiated simply by the base piperidine which leads to the formation of hyperbranched poly(aroylarylene)s with perfect 1,3,5-regioregularity and a high degree of branching (from 78 to 100%) in high yields (up to 99%) and with molar masses $\bar{M}_w$ up to 30 000 g mol^{-1}.[56,57] Here, bis(aroylacetylene)s linked by rather flexible units, e.g. –O(CH$_2$)$_{12}$O–, were employed and the highest degree of branching was achieved in tetralin as proven by spectroscopic means from the disappearance of any remaining alkyne units. These polymers, again fully soluble in common organic solvents, showed high photosensitivity and were readily photocrosslinked to give photoresist patterns with nanometer resolutions. This demonstrates nicely the high potential of cycloaddition reaction in the synthesis of new dendritic polymers with potential use in high performance applications.

In addition to the [2 + 2 + 2] cyclotrimerization reaction being able to be used to prepare branched polyphenylenes it can also allow also to combine effectively perfectly branched monodendrons when suitable units are incorporated as the focal point. Thus, Fréchet and Hecht[58] incorporated acetylenic focal units into polyaromatic ether dendrons (so-called Fréchet-type dendrons) which then allowed to combine three of the dendrons in toluene under cobalt catalysis via the [2 + 2 + 2] cycloaddition reaction by the formed benzene unit (Scheme 7). Even though the yields dropped to 36% when higher generation dendrimers were used, due to the very clean trimerization reaction only the desired products were obtained, which could easily be separated from the unreacted dendrons.

The high versatility already demonstrated so far for the [4 + 2] and [2 + 2 + 2] CA reactions for the preparation of perfectly branched dendrimers as well as hyperbranched or highly branched polymers combined with the interesting property profile of the resulting products, e.g. the combination of high rigidity and high content of aromatic units (leading to high thermo- and chemostability) with easy processibility, allows the assumption that we will see more interesting polymer architectures produced by this approach.

Complex dendritic architectures by 1,3-dipolar cycloaddition reactions

Whereas the above outlined Diels–Alder reactions and [2 + 2 + 2] cyclotrimerization reactions are specially well suited to prepare highly aromatic hydrocarbon structures which exhibit high shape-persistancy and usually a more non-polar character, the 1,3-dipolar cycloaddition reactions offer the possibility to prepare complex heterocycles and also to introduce polar functionalities. In addition, depending on the reaction mechanism and the reaction conditions, a high stereocontrol is possible and the formation of specific intermediates may allow to influence the degree of branching. Usually very high yields can be achieved, however, this high reactivity of the starting compounds can also lead to adverse side reactions between the 1,3-dipoles such as dimerizations and re-arrangements (e.g.
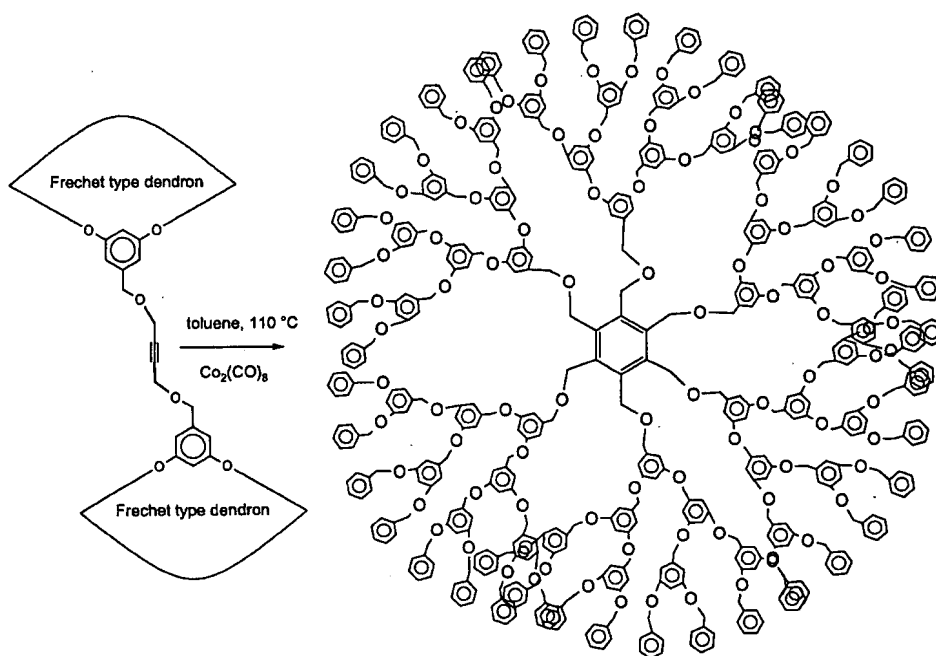
Scheme 7 Dendron core coupling through [2 + 2 + 2] cycloaddition reaction.[58]

sigmatropic shifts), and therefore, the reaction conditions have to be selected carefully.

So far only two types out of the large variety of possible 1,3-dipolar or (3 + 2) cycloaddition reactions have been employed for the preparation of dendritic polymers. One is the reaction of organic azides with alkynes forming a triazole ring, which experienced an enormous revival in many fields of polymer science after Sharpless[20,21] and others[59–61] pointed out that under suitable copper catalysis complete control of the regio-selectivity can be achieved. This, in combination with easy accessibility of the monomers, very high yields, and the insensitivity of the cycloaddition reaction towards many functional groups which led to the term "click chemistry",[20,21] is the base for the huge success of this CA reaction.

The other approach used so far for the preparation of dendritic structures is based on the so-called "criss-cross" cycloaddition of bisazines[62] which always react in a two step dual (3 + 2) cycloaddition *via* an instable azomethine imine 1,3-dipole intermediate (Scheme 8).[63]

"Criss-cross cylcoaddition"

This reaction was used already by Stille and Anyos[12] and later by Maier and co-workers[64,65] for the synthesis of a variety of linear heterocyclic polymers, some based on hexafluoro-acetone azine which is highly reactive and permits polymerizations at room temperature with high regioselectivity.[66] Maier, Voit and co-workers[67,68] extended the use of the criss-cross cylcoaddition reaction to the formation of hyperbranched polymers. For that, an AB$_2$ monomer containing the bisazine moiety (B$_2$ unit) as well as a maleimide function (A unit) was designed and synthetically realized. Due to the special features of that reaction and the instable azomethine imine intermediate it was possible to achieve hyperbranched polymers without any linear units and therefore featuring a degree of branching of 100%. In the first reaction of the maleimide with one of the azine units an instable azomethine imine cyclic system is formed as a linear unit which can only go forward by reacting with the second azine to form a stable tetracyclic system or to go backwards to the starting material. Since the linear units are not stable, they cannot be found in the final product and

azomethine imine: unstable!
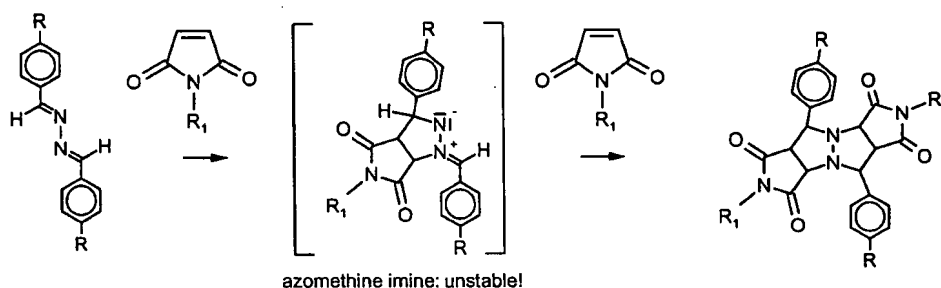
Scheme 8 Formation of a tetracyclic system by 1,3-dipolar cycloaddition reaction of bisazines with maleimides passing through an instable azomethine imine intermediate.
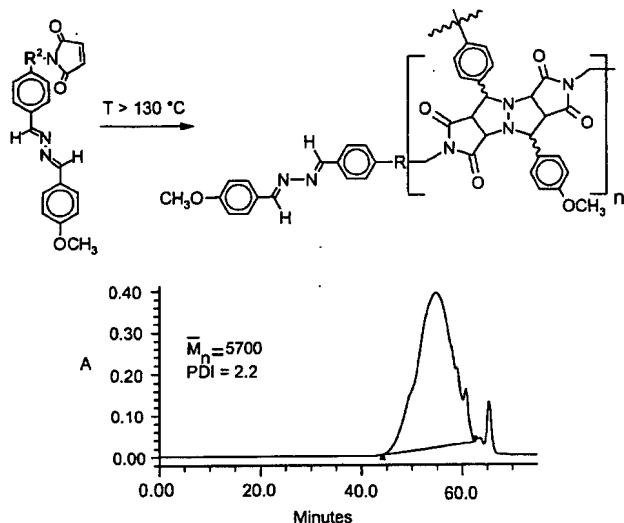
Scheme 9 Hyperbranched polymers with a degree of branching of 100% prepared by 1,3-dipolar cycloaddition of a bisazine-maleimide AB_2 monomer: GPC proves the formation of broadly distributed hyperbranched oligomeric products with $\bar{M}_n$ about 5000 g mol^{-1} (according to calibration with linear standards) (adapted from ref. 67).

only bisazine terminal units as well as tetracyclic fully dendritic units were determined leading to a formal DB of 100%. These structural features and thus, the absence of any linear units could also be proven by detailed NMR analysis and by the use of model compounds despite the rather high number of isomers found in the tetracyclic systems.[69]

Nevertheless, irregularly branched products of molar masses $\bar{M}_w$ up to 10 000 g mol^{-1} and polydispersities around 2.5 (Scheme 9) were isolated and certainly qualified as "hyperbranched".[67] This result is similar as the one reported for the regioselective cyclotrimerization[56] and also Smet et al.[70] were also able to prepare hyperbranched polymers with a degree of branching of 100%, but they used the acid catalyzed condensation of isatin with aromatic compounds—no cycloaddition reaction—leading to hyperbranched polyaryleneoxindoles.

"Click chemistry"

In the field of 1,3-dipolar cycloadditions a very flexible reaction is the addition of organic azides to internal and terminal triple bonds forming triazole rings with the excellent potential to prepare a variety of perfectly branched dendrimers. In addition, it allows for extremely versatile modification reactions, provides the chance for unusual architectures by "clicking together" building blocks and even leads to totally new hyperbranched polymers. This 1,3-dipolar cycloaddition reaction, described first by Huisgen et al.[18] but promoted recently as click chemistry by Sharpless and co-workers,[20,21] was explored very successfully in dendrimer synthesis and functionalization.[71,72] Here one should note, that the thermal cycloaddition reaction allows only minimal control of the regiochemistry and therefore two regioisomers are formed via syn and anti approaches. However, when terminal alkynes are employed, Sharpless and co-workers[21] and Tornoe et al.[60] pointed out that the anti-regioisomer is formed solely using a copper-mediated CA reaction. Unfortunately, this stereocontrol can not be achieved when internal triple bonds are involved in the reaction.

Linear poly(triazole)s have been prepared by Baldwin and co-workers.[73] as early as 1966, but more recently, after "click chemistry" became so fashionable, the 1,3-dipolar CA was also used to postmodify linear polymers having pendant alkyne groups or to combine two polymer segments via end group "clicking" to prepare block copolymers (examples, see refs. 74–77).

Dehaen and co-workers[71] were among the first to use the 1,3-dipolar CA reaction for a convergent approach towards heterocyclic dendrimers. First, the heterocyclic triazole ring was only introduced in the final step when the Fréchet type polyaromatic ether dendrons, which were modified with an acetylenedicarboxylate moiety, were finally coupled to a triazide (Scheme 10). The reaction was carried out at 70 °C for several days to achieve complete conversion as proven by spectroscopic means. Nevertheless, the problem of incomplete
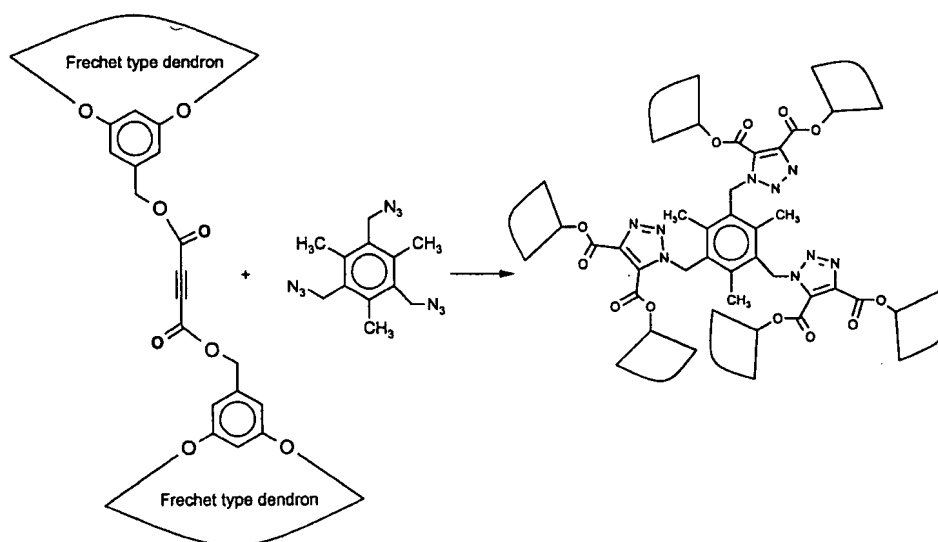
Scheme 10 Attachment of Fréchet type dendrons with an acetylenic focal unit to a trivalent azide core by 1,3-dipolar cycloaddition reaction.[71]
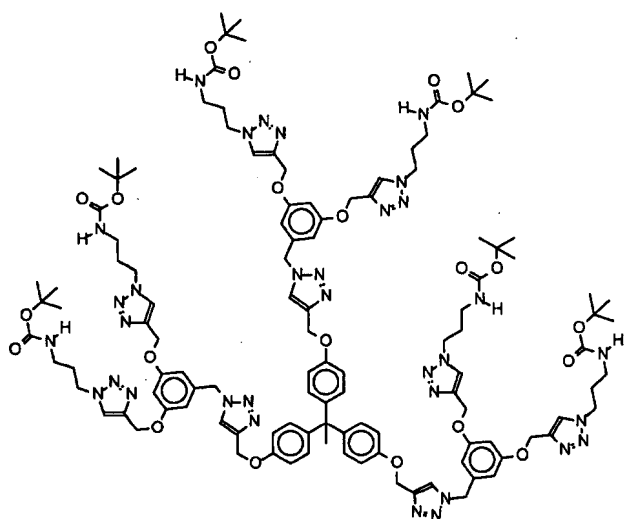
Scheme 11 End-functionalized poly(triazole) dendrimer of generation 1 prepared by "click chemistry".[72]

reaction occurred when dendrons of the third generation were used probably due to steric hindrance.

The incorporation of the triazole rings within the dendrons was reported also by the same group.[71] For that, diethylene glycol spacers between a peripheral azide and a protected core alkyne moiety were introduced. The azide was then reacted with acetylenecarboxylate ester resulting in the formation of an internal triazole ring and peripheral ester groups. After deprotection the focal alkyne unit was then again reacted with a hexafunctional azide core leading to the desired dendrimers.

Lee et al.[78–82] reported also recently a "convergent" type synthesis of dendrimers, symmetric ones and unsymmetric ones, by coupling of suitable alkyne focal units of dendrons to azide cores or vice versa, similar as described by Dehaen and

co-workers[71] (Scheme 10) demonstrating the high versatility. But clearly, the potential given by the combination of click chemistry and dendritic structures is marvellously and extensively demonstrated by Hawker and co-workers,[72,83,84] Sharpless and co-workers[85] and Frechet and co-workers.[86] First, the 1,3-diploar cycloaddition reaction of azides with terminal alkynes was used directly to build up dendrons of different generation by a convergent[72] and a divergent[83] approach. Secondly, terminal alkyne groups in the periphery could be modified easily with readily available functional azides for the introduction of a broad variety of functional end groups including unprotected acid and alcohol groups, protected amines, polar ethylene glycol units, sugar units, nucleosides, dyes, bulky adamantyl groups, and so on.[72,83,84,86] Scheme 11 is presenting a low generation simple example out of those vast structures reported.

It is important to note that in this case a relative simple catalytic system was involved by using $CuSO_4$ and sodium ascorbate which create the necessary Cu^I species. The reaction can be carried out in any kind of polar solvent including water, impurities within the solvent or the monomers are usually of no concern, the reaction is quantitative and purification is easily achieved due to the absence of any side products. When the solubility of the starting components or the products was not high enough in water or alcohols, THF could be employed as solvent using $(PPh_3)_3CuBr$ as catalyst system and the efficiency of the reaction could be further improved when microwave heating was applied.[83,84] This shortened also dramatically the reaction times to sometimes only a couple of minutes. These features are ideal in dendrimer synthesis.

Not only dendrimer build up and end group post-modification was successfully demonstrated but also the attachment of a G2 and even a G3 dendron to a G4 dendrimer having alkyne groups in the periphery in a divergent approach (Scheme 12).[84] Here, again microwave irradiation proved very efficient, thus

G4 + G2

$$\xrightarrow[\substack{Cu(PPh_3)_3Br \\ DIPEA\,/\,THF}]{}$$ **G6-Dendrimer**
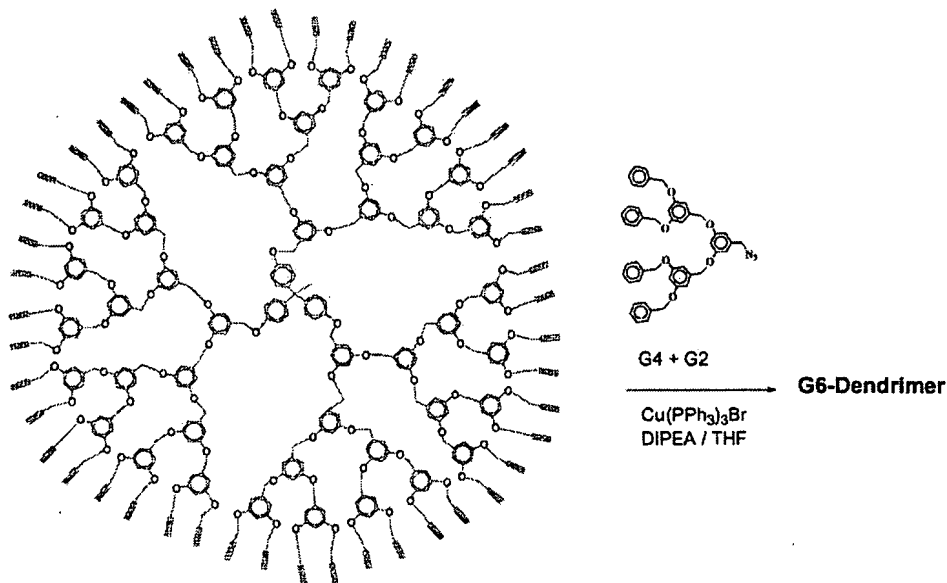
Scheme 12 Preparation of a G6 dendrimer via grafting of G2 azide functionalized dendrons onto an alkyne modified G4 dendrimer (adapted from Hawker and co-workers).[84]
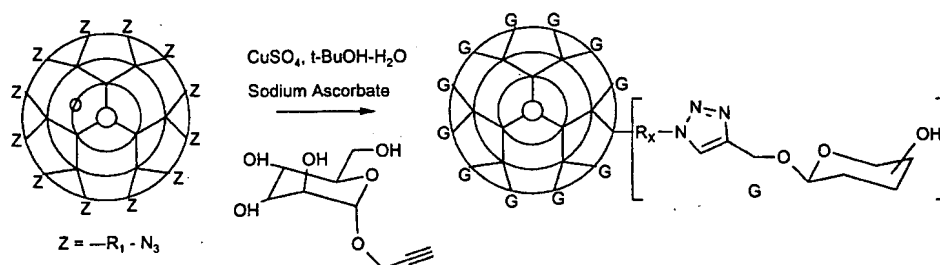
Scheme 13 Postmodification of azide modified dendrimers with unprotected alkyne functionalized sugar moieties leading to glyco-dendrimers (adapted from Riguera and co-workers[89]).

even for these very bulky reagents conversions of 95% (G2) and 75% (G3) could be achieved.

The possibility to have full control of the structure due to the highly efficient CA reaction under very mild reaction conditions and thus, to combine differently modified dendrons as building blocks like in a "Lego™" playground as well as to introduce a certain functionality selectively only to part of a dendrimer structure allows now to build up very complex and highly multifunctional structures. This is demonstrated again nicely by Hawker, Sharpless and co-workers when they produced multivalent, asymmetrical dendrimers containing e.g. 16 mannose units and two coumarin chromophores.[85]

Liskamp and co-workers[87] could show that the microwave assisted synthesis of multivalent dendritic peptides involving click chemistry also works highly efficient. Thus, bioactive oligopetides and cyclic peptides could be functionalized with azide groups and were then linked in high yields to alkyne end-functionalized dendritic structures in THF–water (1 : 1) at 100 °C (microwave irradiation, 10 min) in the presence of CuSO$_4$/Na-ascorbate. Similarly, a variety of azido carbohydrates, protected with benzyl or acetate groups and containing azide moieties, were coupled to alkyne functionalized dendrons (yields >90%) in DMF, under microwave irradiation at 80 °C for 20 min.[88] This approach towards highly biocompatible and even bioactive dendritic molecules was further expanded by Riguera and co-workers,[89] who demonstrated that also unprotected alkyne modified carbohydrate units can be easily linked to dendrimers having azide units in the
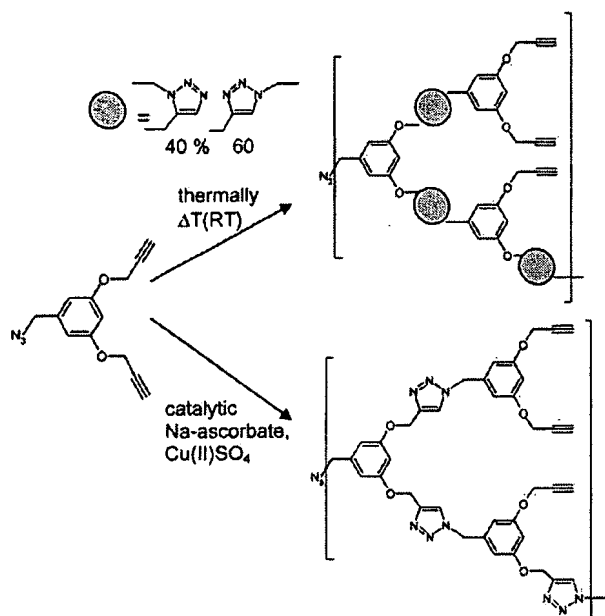
periphery (Scheme 13). However, one problem not dressed in this area so far, might be residual copper within the products which needs to be fully removed before any biomedical application can be addressed.

Besides bioactive components, also other functional units can be easily incorporated into dendrimers via click chemistry postmodification, e.g. recently, ferrocenyl units were incorporated resulting in interesting senor materials[90] and specific electron transfer was studied in fullerene-ferrocene-dendrimer assemblies.[91]

The preparation of dendritic-linear hybrids using click chemistry has attracted also much attention recently. Thus, Fréchet and co-workers[92] used polyaromatic ether dendrons with azide focal units to decorate effectively poly(vinylacety-lene) with pendent dendrons. At this point, only dendrons up to third generation could be attached in quantitative yield after long reaction times (still under conventional conditions). For higher generations steric hindrance was encountered. However, AFM proved already that the polymers decorated with G3 dendrons exhibit rigid rod like structures. In order to overcome the problem of steric hindrance close to the backbone of the linear polymer chain, a new approach was utilized: the coupling of dendrons on dendrons coupled to a linear polymer leading to doubly-dendronized linear polymers (Scheme 14).[86] For that poly(hydroxystyrene) was divergently dendronized with a dendritic poly(aliphatic ester) based on bishydroxypropionic acid up to generation 3. Then, post-modification with pentynoic acid was achieved providing
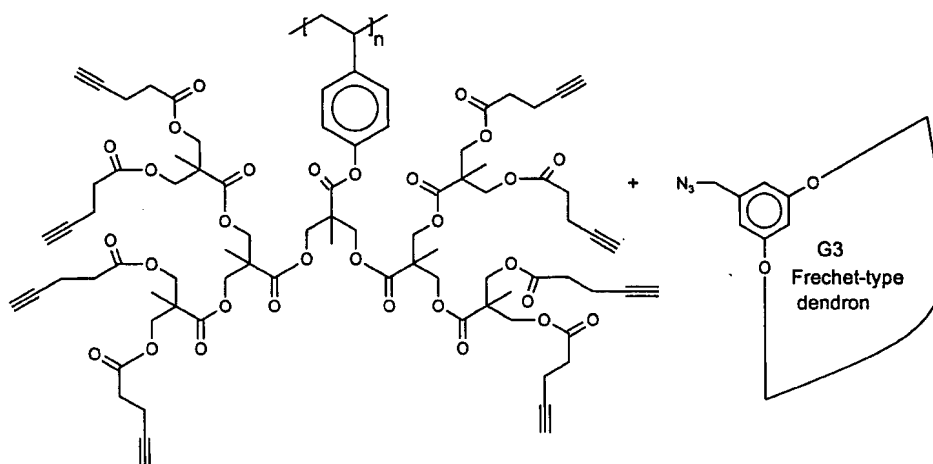
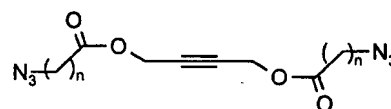**Scheme 14** Doubly dendronized polymers via click chemistry (adapted from Fréchet and co-workers).[86]

Scheme 16 AB$_2$ bisazide ester monomer described by Smet *et al.*[95] for the synthesis of hyperbranched poly(triazole)s *via* thermal 1,3-dipolar CA.

Scheme 15 Preparation of hyperbranched poly(triazole)s *via* Cu^{I} mediated and thermal 1,3-dipolar CA of 3,5-bis(progargyloxy)benzyl azide.[94]

terminal alkyne groups on that dendronized polymer. Click chemistry was applied to link G3-azide dendrons to theses predendronized molecules finally producing a modification of the linear polymers with G6 dendrons (Scheme 14). A nearly quantitative dendronization of each repeating unit was assumed from GPC and spectroscopic evidence.

A new methodology for the preparation of well-defined core–shell nanoparticles was also developed employing click chemistry and dendrimers.[93] For that amphiphilic poly(acrylic acid)-*b*-poly(styrene) block copolymers had been assembled into micelles and were partially functionalized within the corona with alkynyl groups. Subsequently, a divergently grown first generation dendrimer with terminal azide functions was successfully employed as a multivalent crosslinker for the shell of the micelles, allowing the preparation of a very robust nanoparticle with some reactive alkynes in the outer shell which are suitable for further functionalization.
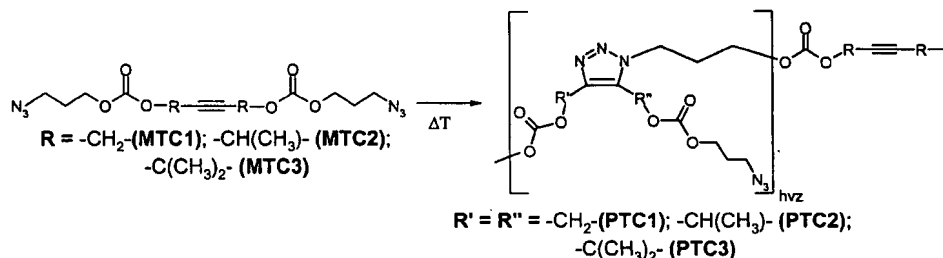
As one can expect, the 1,3-dipolar CA reaction of azides and alkynes also entered the field of hyperbranched polymers. The synthesis of novel hyperbranched poly(1,2,3-triazole)s *via* 1,3-dipolar cycloaddition was successfully achieved with the AB$_2$ monomer 3,5-bis(propargyloxy) benzyl azide, having an azide group as A-unit and two terminal triple bonds as B-units

(AB$_2$-bisalkyne).[94] The polymerization of the AB$_2$-bisalkyne was either conducted *via* thermal polymerization or under copper(I) catalysis. The latter leads to insoluble products under the chosen reaction conditions (room temperature), probably due to the very high molar masses achieved and maybe also due to the occurrence of side reactions. However, full stereocontrol of the formed triazole units was maintained and only 1,4-substitution in the triazole ring as well as a degree of branching of 50%, which is typical for a random and ideal AB$_2$ condensation, was confirmed by ^{1}H and ^{13}C by (HR-MAS) NMR spectroscopy of the swollen samples. By autopolymerization at room temperature fully soluble products of high molecular weight could be obtained from the AB$_2$-bisalkyne but the regioselectivity was lost (Scheme 15). The 1,4- and 1,5-substitutions in the triazole ring were found in a 64 : 36 ratio.

Bisazides with internal triple bonds as AB$_2$ monomers for the synthesis of hyperbranched poly(trizaoles) have been independently developed by Voit and co-workers[94] and Smet *et al.*[95] As mentioned earlier, the high stereocontrol achieved by the Cu^{I} catalyzed click chemistry does not apply for substituted alkynes. Thus, only a classical thermally induced 1,3-dipolar CA reaction will occur leading again to a mixture of 1,4- and 1,5-substituted triazoles. Smet *et al.*[95] studied some ester-alkyne-diazides (Scheme 16). Here, only oligomers could be obtained from the AB$_2$ monomer with $n = 1$ and the products showed a tendency to gel after storage, probably because of some decomposition of the azide groups. Thus, subsequently the azide end groups were transformed into the corresponding amines and further acylated with stearyl chloride which led to a fully soluble product. However, purification and isolation of all obtained oligomers proved difficult due to their oily appearance. In a further step, it was tried to produce an AB$_2$ monomer with an more activated acetylenedicarboxylate unit. Unfortunately, the still protected precursor of the resulting monomer containing a dibromofumaric ester unit started already to polymerize during transformation in the acetylenedicarboxylic ester in refluxing THF and rapid crosslinking of the materials was observed allowing only the observation of some small soluble oligomers by NMR.

R = -CH$_2$-(MTC1); -CH(CH$_3$)- (MTC2); -C(CH$_3$)$_2$- (MTC3)

R' = R" = -CH$_2$-(PTC1); -CH(CH$_3$)- (PTC2); -C(CH$_3$)$_2$- (PTC3)

Scheme 17 Synthesis of hyperbranched poly(triazole)s PTC1–3 from the AB$_2$ bisazide carbonates MTC1–3 *via* thermal 1,3-dipolar CA.[94]
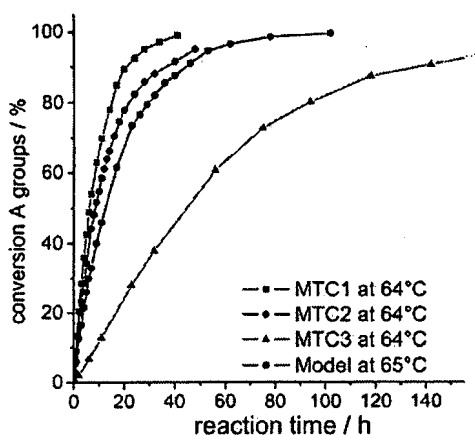
Fig. 1 Dependency of the conversion of A groups on the reaction times for the different AB_2 bisazide carbonates MTC1–3. The model reaction is based on the addition reaction of 3-azidopropanol to 1,4-diacetoxybut-2-inyl (adapted from ref. 96).

Voit and co-workers[94] were able to achieve fully soluble products by low temperature (45 °C) autopolymerization in bulk even of high molar mass ($\bar{M}_n$ = 10 000 g mol^{-1}) from the AB_2 bisazido monomer MTC1 shown in Scheme 17.

Full structural analysis is complex due to the large number of possible isomeric structural units, but the highly branched structure could be verified in the products and a degree of branching of 50% can be assumed. The resulting product bears a large number of highly reactive azide groups which can give rise to a large spectrum of further end-group modification. Here, it is most suitable to use the reaction with functional terminal alkynes again for the postmodification due to the easy and highly efficient reaction. By this it was possible to introduce a variety of polar and unpolar end groups which allow firstly to avoid any side reactions of the azide units and secondly, to adjust the polarity of the material *e.g.* in order to achieve better solubility in unpolar solvents.[96] Polymers based on MTC1–3 have aliphatic carbonate groups in their structure. Aliphatic carbonates are known to decompose readily at temperatures around 200 °C. This makes these hyperbranched polymers to promising materials *e.g.* for the use as porogens for the preparation of nanoporous materials but this also requires to find a suitable solvent for both, porogen and matrix material, for the successful preparation of homogenous films.[96]

In more extended studies[96] it was finally possible to optimize the reaction conditions, and also soluble products could be obtained for MTC2 and MTC3. The reactivity of these two compounds is significantly lower than the one of MCT1 as it was found in some kinetic studies (Fig. 1) carried out at 64 °C. For MTC3 the reaction temperature should not be increased above 45 °C due to the high thermolability of the carbonate units and thus some degradation was observed during the kinetic experiment at 64 °C. However, at 45 °C reaction times of up to four weeks had to be employed to achieve high molar mass products. The evaluation of the data achieved at 64 °C for first- and second-order kinetics showed, that in this AB_2 CA reaction none of these plots showed ideal linear behavior. It looks like that in the beginning of the reaction the expected
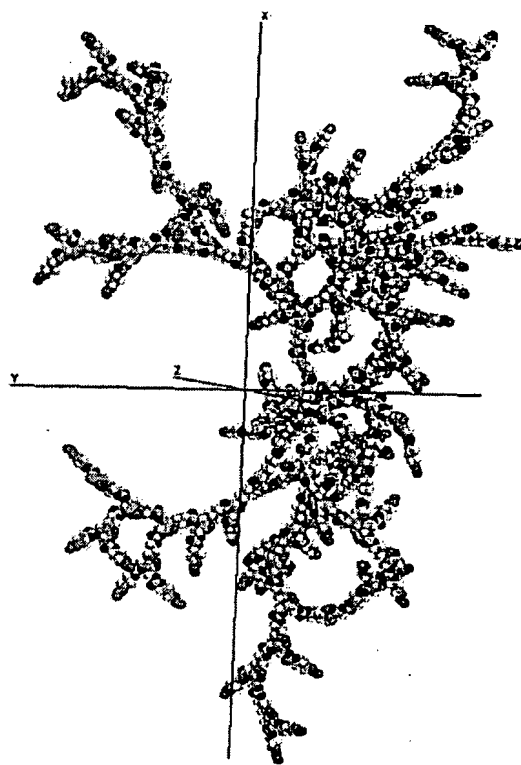

Fig. 2 Molecular modeling result for PTC1 (in vacuum) having a degree of polymerization P_n = 110 (dimensions: x = 19 nm, y = 13 nm, z = 10 nm) (adapted from ref. 96).

second order dependency was valid and later on a first-order dependency took over. Detailed evaluation of this behavior and also a very close study of the accompanying development of the molar masses and the polydispersity led to the conclusion that internal cyclization takes place to a significant proportion at high conversion of functional group. It has to be noted that during these studies it could be verified that soluble products with $\bar{M}_n$ up to 38 000 g mol^{-1} and $\bar{M}_w$ = 100 000 g mol^{-1} (MALLS detection) can be produced from MTC1. Fig. 2 exemplifies a molecular modeling result for the hyperbranched polymer PTC1 with a degree of polymerization of 110 showing a highly branched but still relatively open, irregular structure with dimensions of 19 × 13 × 10 nm.[96]

Since it is still rather difficult to produce hyperbranched polymers *via* the AB_2 approach due to the high reactivity of the functionalities and thus problems of monomer isolation and the danger of premature polymerization, it looks like the 1,3-dipolar CA of alkynes with azides by the A_2 + B_3 approach will enter the field of technical branched products more easily, mainly because suitable A_2 and B_3 monomers are relatively easily available. Here, Finn and Diaz[97] reported on the synthesis of linear but also branched polymers prepared by di, tri- and tetra-alkyne and azide monomers like the combination of tripropargylamine (B_3) and (dihydroxymethyl)(diazidomethyl)amine (A_2). The products obtained had very good adhesive properties and significantly outperformed commercial adhesives.

Outlook

Cycloaddition reaction entered, with a few exception, only recently the field of dendritic polymers. Nevertheless, due to the resulting high yields, high selectivity, very clean reactions and often the absence of any side reaction, they proved already extremely versatile and ideal for the effective dendrimer synthesis as well as highly efficient for the post-modification of dendritic highly functional macromolecules. Especially two types of reactions are very promising: the Diels–Alder reaction of cyclopentadienone with alkynes leading to a large variety of otherwise very difficult to realize polyarylene dendrimers and the 1,3-dipolar cycloaddition reaction of azides with alkynes. The later allows not only the preparation of fully new dendritic structures, it is also very efficient in order to introduce complex functionality into dendritic structures.

The so far limited variety of CA reaction used in the synthesis of dendritic polymers points also to the rather unlimited potential which still can be explored. Necessary requirements, however, are reasonable good availability of the needed starting compounds as well as sufficient stability of the reactive functionalities used in the CA reactions. With this in mind, it can be expected that the field will expand very rapidly leading to beautiful new dendritic architectures but also allowing for very effective synthesis procedures which might promote the commercialization of dendritic molecule for high-tech applications.

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1,3-Dipolar Cycloadditions of Azides and Alkynes: A Universal Ligation Tool in Polymer and Materials Science

Jean-François Lutz*

In 2001, Sharpless and co-workers introduced "click" chemistry, a new approach in organic synthesis that involves a handful of almost perfect chemical reactions. Among these carefully selected reactions, Huisgen 1,3-dipolar cycloadditions were shown to be the most effective and versatile and thus became the prime example of click chemistry. Hence, these long-neglected reactions were suddenly re-established in organic synthesis and, in particular, have gained popularity in materials science. The number of publications dealing with click chemistry has grown exponentially over the last two years. The Minireview discusses whether click chemistry is a miracle tool or an ephemeral trend.

1. Introduction

Most of the major scientific challenges of the 21st century require nanomaterials with a high degree of structural order and defined properties. To meet this demanding level of complexity often necessitates the design of novel organic or inorganic molecules using sophisticated multistep experimental procedures. In this context, the discovery and selection of simpler and universal synthetic methods is essential. Indeed, the palette of chemical reactions developed within the last 100 years is extremely broad; however, many of these established reactions show significant limitations within a contemporary scientific context.

Selecting the most versatile chemical tools is the essence of "click" chemistry, an appealing concept proposed by Sharpless and co-workers.[1] Click chemistry is not a scientific discipline but rather a synthetic philosophy inspired by the simplicity and efficiency of the chemistry that takes place in nature. Indeed, highly complex biological systems rely on a modest library of monomers linked together by a few, but efficient, organic reactions. Similarly, the objective of click

chemistry would be to establish an ideal set of straightforward and highly selective reactions in synthetic chemistry. For instance, the archetypal example of click chemistry is undoubtedly the copper-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes.[2,3,6,7,93] In the absence of an appropriate catalyst, this reaction is usually quite slow as alkynes are poor 1,3-dipole acceptors. However, in the presence of copper(I), which can bind to terminal alkynes, cycloaddition reactions are dramatically accelerated, regioselective, and highly efficient (yields are often above 95%). Moreover, the copper-catalyzed azide–alkyne cycloaddition (CuAAC) can be performed in various solvents (including water) and in the presence of numerous other functional groups.[6,7]

Although click chemistry was initially postulated as a general concept for organic synthesis, this strategy also has an enormous potential in materials science.[8,9] The first report that illustrated this point appeared in mid-2004 by Hawker, Fokin, Sharpless, and co-workers.[10] Afterwards, the popularity of click chemistry within the materials science community grew considerably and has been, in particular, significantly boosted by the influential works of Hawker, Fréchet, and Finn.[10–24] As a result, the number of publications in this field has increased dramatically within the last two years. The target of this Minireview is to analyze and categorize this recent scientific trend.

[*] Dr. J.-F. Lutz
"Nanotechnology for Life Science" Research Group
Fraunhofer Institute for Applied Polymer Research
Geiselbergstrasse 69, 14476 Potsdam (Germany)
Fax: (+49) 331-568-3000
E-mail: lutz@iap.fhg.de

2. Design of Novel Polymer Materials

Huisgen cycloadditions have been recently investigated as ligation tools for synthesizing linear polymers, dendrimers,

and rotaxanes.[10,11,14,22,25–27] Such structures containing multiple triazole or tetrazole repeating units constitute a novel class of macromolecules with potentially interesting properties. Hawker, Fokin, Sharpless, and co-workers explored first the CuAAC of various molecular building blocks for the convergent synthesis of dendrimers.[10] Overall, this method was found to be a straightforward strategy for the large-scale synthesis of triazole-based dendrimers. Shortly after, Finn and co-workers studied the click cycloaddition of azide- and alkyne-functionalized monomers for the preparation of either linear polymer chains (Scheme 1, 1) or three-dimensional
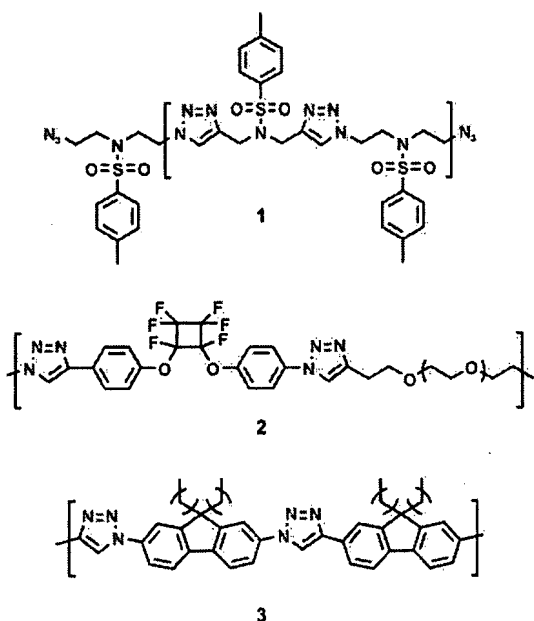
Scheme 1. Examples of linear polymer structures obtained by click polycycloaddition of azide- and alkyne-functionalized monomers.[11,25,27]

polymer networks.[11] The latter were investigated as novel adhesives for copper surfaces, as triazole rings have a strong ability to coordinate transition metals.

Novel conjugated polymers may also be prepared using CuAAC. The groups of Reek and Bunz both reported the

Jean-François Lutz studied chemistry at the Université Pierre et Marie Curie (Paris VI) and completed his PhD at Montpellier under the supervision of B. Boutevin. He then spent two years as a postdoctoral fellow with K. Matyjaszewski at Carnegie-Mellon University (Pittsburgh, USA). Since late 2003, he leads the Nanotechnology for Life Science research group at the Fraunhofer Institute for Applied Polymer Research (Potsdam-Golm, Germany). His interests include polymer bioconjugation, stimuli-responsive polymers, and the self-organization of macromolecules in aqueous media.

preparation of poly(fluorenylene-triazolene) through the reaction of diazidofluorene monomers with various diynes (Scheme 1, 3).[25,28] Interestingly, Bunz and co-workers additionally showed that these monomers could be locally polymerized, in the absence of a copper catalyst, by using a heated atomic force microscopy tip.[28]
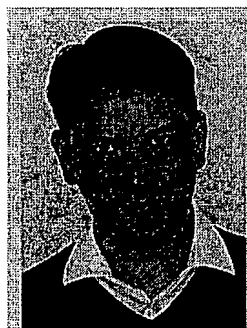
Qing and co-workers prepared poly(alkyl aryl) ethers containing 1,2,3-triazolyl and perfluorocyclobutyl units (Scheme 1, 2) through click polycycloaddition.[27] These novel macromolecules exhibited a rather interesting thermal stability and melting fluidity. In comparison, polymers containing tetrazole units were found to be less thermoresistant. Matyjaszewski and co-workers synthesized polymers containing multiple tetrazole side groups by treating well-defined polyacrylonitrile precursors with sodium azide.[29] Their study indicated that tetrazole functionalities start to decompose at temperature as low as 120°C.

Overall, Huisgen cycloadditions appear to be straightforward and efficient reactions for building novel polymer structures. However, so far, synthesis has been the prime focus of the aforementioned reports. Further studies are needed to assess comprehensively the physical behavior of these novel triazole- or tetrazole-containing polymers. Nonetheless, some of these early reports already suggested some interesting properties (e.g. solubility, swelling, and metal adhesion).
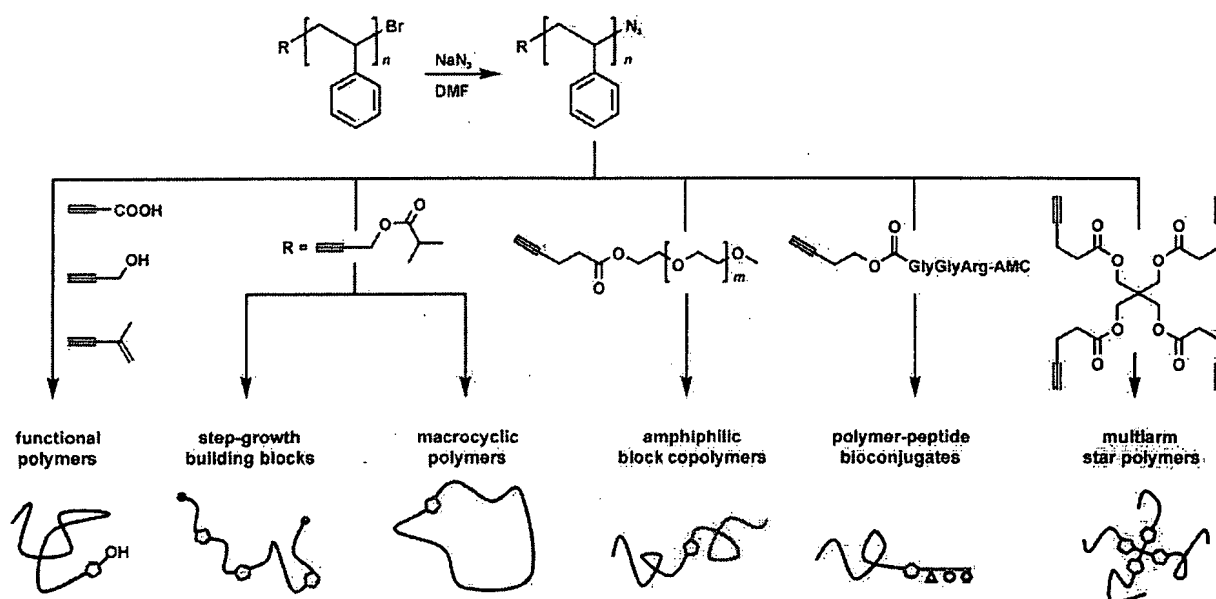
3. Macromolecular Engineering

Macromolecular engineering can be generally defined as the fabrication of complex macromolecular structures with defined composition, microstructure, functionality, and architecture (e.g. telechelic polymers, block copolymers, macromolecular brushes, stars, and networks) using covalent chemistry approaches.[30] Click reactions, and in particular Huisgen cycloadditions, were recently shown to be extremely versatile tools for such advanced macromolecular design. For instance, during the last two years, the 1,3-dipolar cycloaddition of azide and terminal alkynes has been investigated as a complementary tool for most of the major synthetic polymerization techniques such as cationic or anionic ring-opening polymerization (ROP),[31–34] ring-opening metathesis polymerization (ROMP),[35] polycondensation,[36] conventional free-radical polymerization,[12,37] nitroxide-mediated polymerization (NMP),[13,16] reversible addition–fragmentation chain-transfer polymerization (RAFT),[38] and atom-transfer radical polymerization (ATRP).[21,29,39–55] However, to date, the latter method has involved CuAAC more often than any of the others. Indeed, ATRP is a facile and versatile polymerization technique and therefore probably one of the most employed tools in modern polymer chemistry.[30,56] Nevertheless, the range of macromolecular engineering possibilities of ATRP can be further broadened by click chemistry (Scheme 2).

The first important application of CuAAC in polymer chemistry is undeniably the synthesis of functionalized polymers (either end-functionalized or side-functionalized). The post-functionalization of synthetic polymers is an im-

Scheme 2. Variations on a simple theme: examples of macromolecular architectures recently obtained by click modification of well-defined polystyrene prepared by ATRP.[39,42,44,46,48,51]

portant feature of macromolecular engineering, as many polymerization mechanisms are rather sensitive to bulky or functional groups. For example, telechelic polymers (i.e. polymers with defined chain ends) can be efficiently prepared through a combination of ATRP and CuAAC. The halogen end groups of polymers prepared using ATRP can be easily transformed into azide moieties and subsequently used in Huisgen cycloadditions with functional alkynes (Scheme 2).[40,42,46,53,54] Alternatively, azide- or alkyne-functionalized initiators can also be utilized.[43,44,46]

Polymers with multiple functional side chains have been prepared by CuAAC using precursors built with alkyne-functionalized monomers.[12,16,31,34,36] For example, Fréchet and co-workers constructed dendronized polymers (i.e. linear polymer chains with bulky side dendrons) through cyclo-addition of the side chains.[12] Hawker and co-workers explored this concept even further and reported some very elegant examples of cascade side-chain functionalization of macromolecules (Scheme 3).[16] Azide-containing monomers and related polymer precursors have also been studied for the preparation of macromolecules with functional side groups.[32,35,37,45] However, although efficient, such strategies are experimentally risky (like any approach involving low-molecular-weight azides or macromolecules with a high density of azide groups) owing to the potentially explosive character of organic azides and should be investigated with extreme care.[57,58]

Besides the preparation of functional macromolecules, click reactions were also shown to be very useful tools for building polymers of defined architecture. The first example of architectural control using click chemistry was reported by van Hest and co-workers, who synthesized amphiphilic block copolymers by coupling azide- and alkyne-functionalized
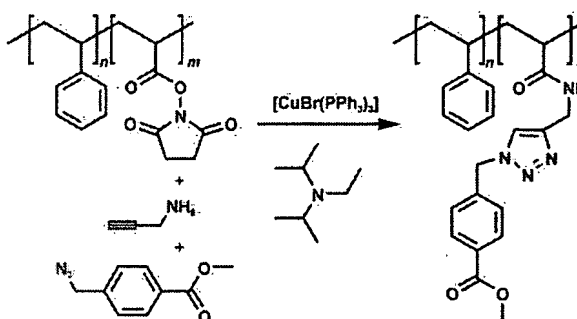
Scheme 3. Example of cascade functionalization (amide formation and cycloaddition) of synthetic macromolecules.[16]

segments (Scheme 2).[44] Such a ligation strategy has been shown to be quite efficient for linking homopolymers of various nature. Grayson and co-workers recently described an elegant strategy for preparing macrocycles (i.e. polymer rings, which are often rather difficult to prepare) based on the self-ligation of well-defined α-alkyne-ω-azido-telechelic polystyrenes (Scheme 2).[51] Additionally, other nonlinear architectures such as stars,[33,48] miktoarm stars,[47,55] graft copolymers,[54,59] and networks[17,21] were constructed using click chemistry. All these studies relied on the Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes, with the exception of the approach reported by Yagci and co-workers for preparing graft copolymers which involved anthracene–maleimide Diels–Alder chemistry.[59]

As mentioned above, CuAAC was explored for preparing triazole-containing dendrimers. Alternatively, Huisgen cyclo-additions were studied for functionalizing the outer shells of

various dendrimers or for linking preformed dendrons of various chemical nature (e.g. polyamidoamine- or polyester-based dendrons).[15,19,60,61] The latter reaction is an interesting route for preparing unsymmetrical dendrimers with distinct hemispheres.[19]

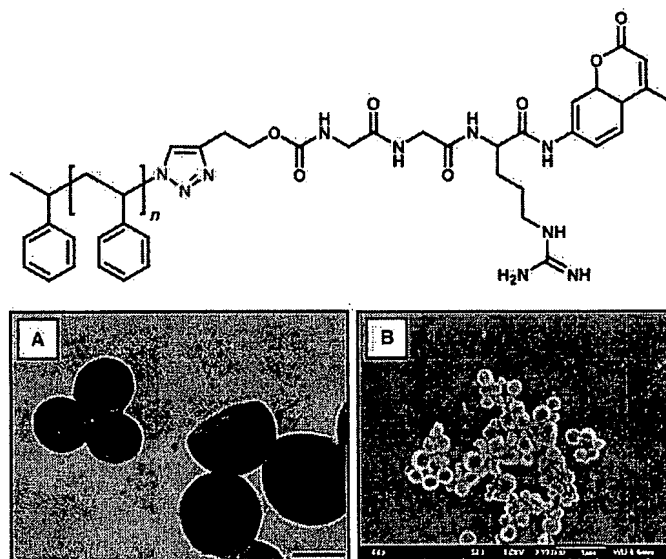## 4. Bioconjugation

An important advantage of Huisgen cycloadditions is undoubtedly their very high degree of selectivity. For instance, the copper-catalyzed reaction of organic azides with terminal alkynes is tolerant to a wide variety of chemical functions.[6] This particular feature makes these reactions particularly attractive for modifying highly functional bio-molecules. For example, CuAAC was investigated for modifying biological polymers such as nucleic acids or polysac-charides. (The strategies for functionalizing biopolymers are conceptually similar to those described above for synthetic polymers.)[62–64] Moreover, the Huisgen cycloaddition of azides and alkynes was shown to be very useful for preparing polymer bioconjugates (i.e. hybrid macromolecules composed of distinct synthetic and biological moieties). For instance, several reports indicated that sequence-defined oligopeptides can be linked to synthetic macromolecules using click ligation.[31,39,53,65] In particular, Nolte and co-workers de-scribed the synthesis and self-assembly in aqueous medium of bio-hybrid amphiphiles composed of a hydrophobic polystyr-ene segment coupled to a hydrophilic oligopeptide (Fig-ure 1).[39] Besides peptide bioconjugation, Huisgen cyclo-additions were also explored for functionalizing linear or dendritic synthetic macromolecules with carbohydrates (e.g. mannose, galactose, fucose, or lactose moieties).[19,50,66]

More complex biological entities such as proteins, en-zymes, viruses, bacteria, and cells may also be transformed using azide–alkyne chemistry.[67–72] For example, Finn and co-workers efficiently modified the surface of the cowpea mosaic virus using CuAAC.[70] Indeed, such reactions can be per-formed under experimental conditions that are compatible with biological environments (e.g. aqueous medium and room temperature). However, such chemical modifications of biological assemblies should be cautiously characterized as many reactants or catalysts may induce denaturation or disassembly.[70] Additionally, Nolte and co-workers reported some interesting examples of protein conjugation (either transport proteins such as bovine serum albumin or enzymes such as lipases) using CuAAC.[39,73] In particular, they reported efficient procedures for linking proteins with manmade materials such as synthetic polymers or inorganic nanoparticles.

Other elegant examples of protein functionalization using CuAAC have been reported by the groups of Schultz and Tirrell.[67,68,72,74] Their modification approach relies on the use of nonnatural amino acids containing azide functions (e.g. *para*-azidophenylalanine, azidohomoalanine, azidonorvaline, and azidonorleucine), which can be incorporated into mutant proteins using either genetic engineering or the metabolic replacement of a natural amino acid by a noncanonical substitute.[67,75] The formed azido-functionalized proteins can be subsequently reacted with various functional alkynes. Such ligations can even be directly performed on cell surfaces if membrane proteins are mutated.[68,72] Moreover, the click strategy of Tirrell and co-workers was recently applied for distinguishing new proteins from old proteins in mammalian cells.[76] In this approach, only the newly synthesized proteins contain azidohomoalanine and can therefore be selectively labeled by an alkyne affinity tag.

Figure 1. Top: Molecular structure of an amphiphilic polymer bioconjugate polystyrene-*b*-oligopeptide synthesized partly by click chemistry. Bottom: Visualization by electron microscopy of its aggregates in water: A) TEM and B) SEM. Reproduced from reference [39] with permission from the Royal Society of Chemistry.

5. Functional Nanomaterials

Highly efficient and selective click reactions have an enormous potential in nanoscience. Indeed, as opposed to molecular products obtained by covalent means, modern nanomaterials are often fragile supramolecular constructions, which cannot be easily purified or isolated. In this context, straightforward in situ reactions are rather valuable in materials science. Thus, the copper-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes, which can be performed at room temperature in multiple solvents and with stoichiometric amounts of reactants, has recently received increased attention from several research groups focusing on material design.

First, CuAAC has been shown to be a versatile tool for functionalizing or cross-linking colloidal objects such as polymer, lipid, or inorganic nanoparticles.[13,77–81] For example, Wooley and co-workers obtained shell-cross-linked polymer micelles by reacting alkyne moieties present in the hydrophilic outer shell of the micelles with azide-functionalized first-generation dendrimers.[13] The groups of Schuber and Kros reported elegant pathways for functionalizing liposomes (i.e. lipid vesicles).[77,79] Both approaches utilized alkyne-functionalized surfactants, which can be incorporated in the lipid bilayers and further reacted with azide-containing molecules. Their studies revealed that the lipid membranes are not damaged during this functionalization process and that only the outer surface of the vesicles are modified.[77,79] Moreover, a few routes have been reported recently for the click functionalization of inorganic nanoparticles.[80,81] In particular, Turro and co-workers coated the surface of maghemite particles with either azide- or alkyne-functionalized ligands, which were shown to be highly versatile platforms for further functionalization.[81] Besides spherical particles, anisotropic objects such as nanotubes were also modified by click chemistry. Adronov and co-workers reported an elegant pathway for functionalizing carbon nanotubes with polymers and thus for promoting colloidal dispersion of the resulting nanotubes in organic solvents (Figure 2).[41]

The azide/alkyne ligation was also studied for preparing various types of bulk materials.[17,18,20,23,82] The groups of Hawker and Hilborn reported the synthesis of poly(ethylene glycol) or poly(vinyl alcohol) hydrogels cross-linked by triazole rings.[17,82] Similar chemistry was used for attaching ligands on gel beads that are utilized in affinity chromatography or electrophoresis.[18,23]

Moreover, CuAAC has been shown to be an unprecedented tool for functionalizing flat surfaces. Pioneers in this area are undoubtedly Collman and Chidsey, who reported several important examples of self-assembled monolayers (SAMs) functionalized by triazole linkages.[84–87] Their work primarily focused on gold surfaces, but was extended by other groups to different types of substrates such as silicon wafers or glass slides.[88,89] Overall, a very wide variety of functional molecules (synthetic or biological) have already been attached to SAMs using an azide/alkyne strategy, thus opening a wide range of opportunities for applications such as molecular electronics, catalysis, or biosensors.[84,85,88–91] Besides the
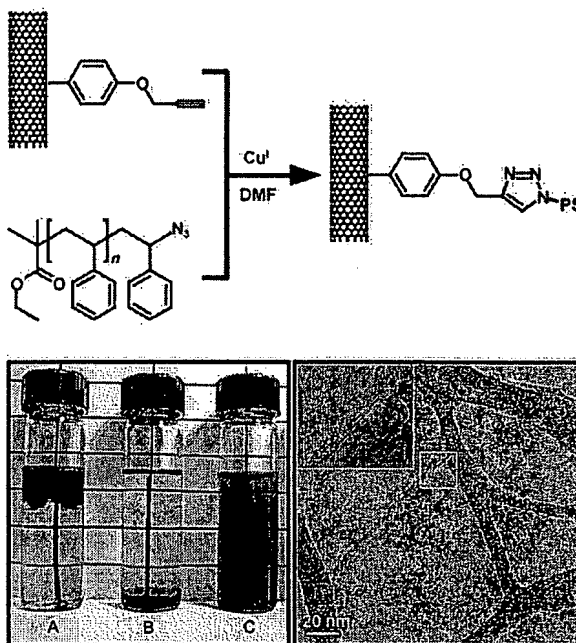

Figure 2. Top: Preparation of polystyrene-modified single-walled carbon nanotubes using click chemistry. Bottom left: THF solutions of pristine (A), alkyne-functionalized (B), or polymer-modified (C) carbon nanotubes. Bottom right: Transmission electron micrographs of the polymer–nanotube organic–inorganic hybrid structures (the inset shows a magnified view of the area within the white square). Adapted from reference [41] with permission from the American Chemical Society.

functionalization of SAMs, the Huisgen cycloaddition of alkynes and azides was also utilized for constructing polymer-modified surfaces. For example, Caruso and co-workers developed a click layer-by-layer technology (i.e. using alternating layers of alkyne- and azide-functionalized polymers) for constructing defined polymer films on either quartz, silicon, or gold surfaces.[38]

Two recent reports described the spatial control of click cycloadditions on flat surfaces by using microcontact printing.[83,92] Lahann and co-workers prepared defined biotin-functionalized patterns with the help of a poly(dimethylsiloxane) (PDMS) stamp inked with a solution of copper sulfate, which catalyzed locally the cycloaddition of an adsorbed alkyne polymer and biotin azide.[92] Alternatively, Reinhoudt and co-workers utilized an alkyne-inked PDMS stamp for creating various functional patterns on azido SAMs (Figure 3).[83] Interestingly, this approach did not necessitate a metal catalyst. Owing to the high local concentration of reactants in the confined regions between the stamp and the substrate, the azide/alkyne cycloaddition occurred spontaneously within a short period of time.

6. Summary and Outlook

Huisgen cycloadditions have become major ligation tools in material science in the past few years and have recently
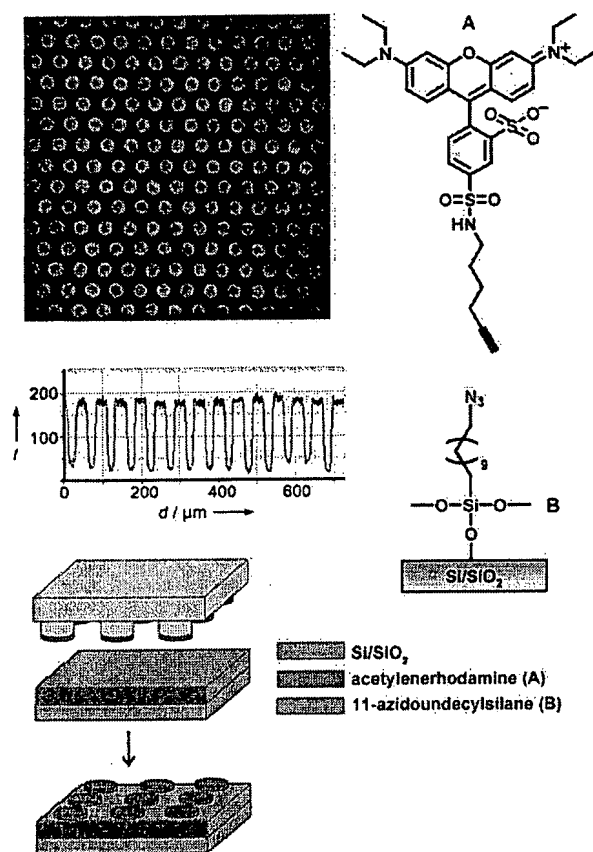
Figure 3. Example of click chemistry by microcontact printing in the absence of a copper catalyst. Top left: Visualization by fluorescence microscopy of the surface pattern (image width is 700 μm). Adapted from reference [83].

been shown to be useful in areas as diverse as polymer science, molecular biology, or nanoelectronics. However, Huisgen cycloadditions and click chemistry should be distinguished. Most of the reports described above used the dipolar cycloaddition of azides and alkynes, but did not necessarily follow the strict concept of click chemistry. Indeed, in many studies, scientific motivations and experimental conditions are often far from those initially promoted by Sharpless and co-workers. However, paradoxically, although the concept of click chemistry has often been misunderstood, it has been validated. Nowadays, Huisgen cycloadditions appear as universal ligation reactions, which compare in versatility with some of the most essential chemical reactions in nature.

Nevertheless, the 1,3-dipolar click cycloaddition of organic azides and terminal alkynes still presents some limitations. For instance, the use of a copper-based catalyst remains a drawback in some delicate applications. Thus, the development of optimized catalytic methods, either metal-free or involving other transition metals, is an important issue in this field. Moreover, the tantalizing concept of click chemistry should not be exclusively limited to Huisgen cycloadditions. The search for alternative versatile click reactions and

ultimately for a complete toolbox of click reactions should press on.
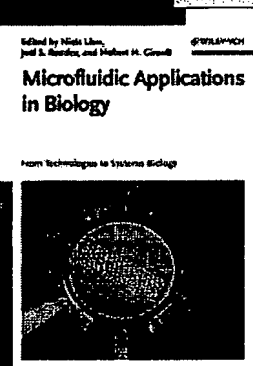
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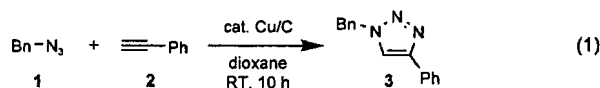[93] V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51–68.

Heterogeneous Catalysis

Heterogeneous Copper-in-Charcoal-Catalyzed Click Chemistry**

Bruce H. Lipshutz and Benjamin R. Taft*

Quick inspection of the 2006 literature already turns up nearly 200 papers related to "click" cycloadditions. Click chemistry is clearly on the rise, and for good reason. It offers an almost unlimited array of inert triazole-containing architectures resulting from Huisgen [3+2] cycloadditions,[1] in particular between high-energy organic azides and terminal alkynes, for example, as shown in Equation (1) (Bn = benzyl). This

$$
\text{Bn}-\text{N}_3 \ + \ \equiv\!\!-\text{Ph} \ \xrightarrow[\substack{\text{dioxane} \\ \text{RT, 10 h}}]{\text{cat. Cu/C}} \ \underset{\mathbf{3}\ \ \text{Ph}}{\text{Bn}-\text{N}\cdot^{\text{N}}\cdot\text{N}} \tag{1}
$$

$$\mathbf{1} \qquad\qquad \mathbf{2}$$

process is significantly accelerated by Cu^I catalysis, independently discovered and published by the groups of both Sharpless[2] and Meldal[3] in 2002. Recently reviewed,[4] copper(II) sulfate (ca. 1%) in an aqueous medium (e.g. $H_2O/tBuOH$) is typically preferred, where the presence of sodium ascorbate (ca. 10%) serves to generate catalytically active Cu^I in situ. Alternative conditions, such as in situ oxidation of Cu^0 or direct introduction of Cu^I salts (usually CuI), have also been used quite successfully.[5] While both

[*] Prof. Dr. B. H. Lipshutz, B. R. Taft
    Department of Chemistry and Biochemistry
    University of California, Santa Barbara
    Santa Barbara, CA 93106-9510 (USA)
    Fax: (+1) 805-893-4120
   E-mail: lipshutz@chem.ucsb.edu
    Homepage: http://www.chem.ucsb.edu/~lipshutzgroup/

reaction partners have been individually coupled under solid-phase conditions, (e.g. on polystyrene),[3,6] examples of click chemistry mediated by a source of heterogeneous copper(I) are rare. One report relies on chelation to potentially labile copper by a polystyryl-based benzylic amine.[7a] Only unhindered, low-molecular-weight, and non-basic nitrogen-containing examples were studied therein, without data quantifying losses of copper from the solid support. Other studies are also limited, based on suspensions of unsupported copper clusters.[7b] Herein, we describe the virtues of copper-in-charcoal (Cu/C) as a simple, inexpensive, and especially general and efficient heterogeneous catalyst for use in this emerging area.

Impregnation of activated wood charcoal (Aldrich, 100 mesh, $53.90/kilo)[8] with Cu(NO₃)₂ in water using an ultrasonic bath leads, after distillation of water and drying, to nanoparticle-sized Cu/C.[9] As both CuO and Cu₂O have been proposed as the species present within a charcoal matrix,[10] the presence of Cu^I suggested that a reducing agent might not be needed. Indeed, upon mixing benzyl azide (1) with phenylacetylene 2 (1:1) in dioxane at room temperature in the presence of 10 mol% Cu/C, cycloaddition was complete within 10 hours. Filtration and solvent evaporation afforded pure triazole 3 regiospecifically and near-quantitatively.

Aromatic alkynes are usually among the more reactive partners, while aliphatic alkynes oftentimes result in significantly slower cycloadditions. Steric hindrance in either substrate can also reduce rates, but with mild heating (60 °C) reactions are driven to completion (Scheme 1).
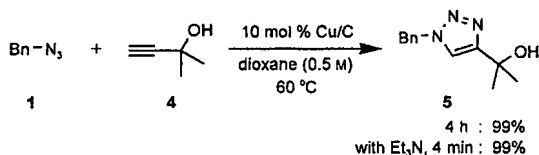
Scheme 1. A representative click reaction under mild heating.

Surprisingly, no bases, ligands,[11] or other additives are required for Cu/C to effectively catalyze these cycloadditions. However, given the likely intermediacy of copper acetylides,[4,5,12] the impact of an amine base was examined. This additive is assumed to aid in both anion formation as well as Cu^I stabilization, thereby significantly increasing rates of triazole formation.[13] Cycloaddition catalyzed by Cu/C in the presence of several different bases led to the unexpected finding, contrary to literature precedent (which encourages use of excess Hünig's base),[14] that one equivalent of Et₃N was particularly effective. Stronger (e.g. 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU)) and weaker (e.g. pyridine) amine bases had marginal impact (<10% conversion under otherwise identical conditions), while lithium tert-butoxide showed no catalytic effect whatsoever. The dramatic role of Et₃N was noted in the case of partners 1 and 4, for example, where the reaction time leading to product 5 was reduced from 4 h to 4 min (Scheme 1).

Several additional examples were carried out using the same conditions: Cu/C (cat.), Et₃N (1 equiv), dioxane, 60 °C

(Table 1). Noteworthy features of this process include a) the regiochemistry (1,4-triazole) of addition follows that anticipated for such Cu-catalyzed reactions;[2] b) outstanding functional group tolerance is observed under these heterogeneous

Table 1: Click reactions catalyzed by Cu/C.[a]

Entry	Alkyne	Azide	t [min]	Triazole	Yield [%]
1	≡—Ph	Bn-N₃	10		99
2	≡—⟍—OH	Bn-N₃	20		97
3	≡—⟨(HO)	Bn-N₃	20		99
4	≡—(CH₂)₃—Cl	Bn-N₃	20		94
5	≡—⟨OH⟩(CH₂)₄	Ph⟍⟍N₃	45		99
6[b]	≡—CH₂-phthalimide	BnN₃	20		96
7	≡—Ph	Ph⟍⟍N₃	30		92
8	≡—(2-pyridyl)	Ph⟍⟍N₃	30		98
9	≡—(cyclohexanol)	adamantyl-N₃	120		99
10[d]	≡—(fluorenol)	adamantyl-N₃	120		97

[a] Run at 0.5 M in dioxane at 60 °C with 5 mol% Cu/C and 1 equivalent Et₃N. [b] Direct comparison to Sharpless' method (88% yield).[2] [c] Product was further purified by silica gel chromatography.

conditions; c) yields are uniformly high; d) generally, reactions are complete within minutes; e) sterically demanding precursors are not precluded from participation; f) heteroaromatics couple smoothly; and g) high-molecular-weight adducts, such as the triazole-containing steroidal derivative 6 ($M_r = 673$), and the precursor to an analogue of coenzyme Q₁₀, 7 ($M_r = 1029$), were both readily formed and isolated in good yields (Figure 1).

Numerous control experiments indicated not only that the Cu^I dispersed within charcoal is essential for catalysis but that Cu/C is also quite robust. Treatment of 1 and 4 with activated charcoal alone, from the same source used to make Cu/C,[8] gave no trace of product 5 (Table 2, entry A). Meticulous drying of these substrates, solvent, and catalyst (including the use of 4-Å molecular sieves) did not noticeably alter the rate
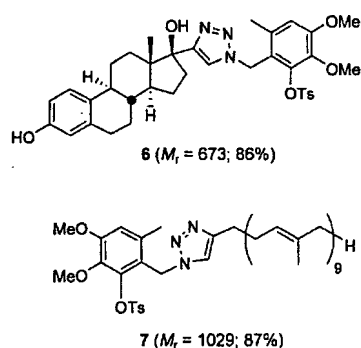
6 (M_r = 673; 86%)

7 (M_r = 1029; 87%)

Figure 1. Examples of higher-molecular-weight adducts. Ts = *para*-toluenesulfonyl.

Table 3: Microwave irradiation in the absence of base.

1,4 isomer only

T [°C]	t	Yield [%]
conventional heating		
23	48 h	65
60	4 h	99
microwave irradiation		
120	10 min	99
150	3 min	99
150 (no catalyst)	3 min	0

Table 2: Assessment of catalyst (Cu/C).

Entry	Conditions	Yield [%]
A	charcoal, (no Cu), 60°C, 1 h	0
B	dry dioxane with 4-Å mol. sieves	99
C	wet, 2:1 dioxane/H₂O	99
D	recycled catalyst in air	99
E	repeat (D), third use in air	99
F	dioxane, room temperature, 6 h	99
G	toluene, room temperature, 2 h	98
H	95 % EtOH, 60 °C, 10 min	98
I	H₂O, 60 °C, 10 min	97

or yield of reaction (Table 2, entry B). At the other extreme, using water as co-solvent, likewise, had no impact (Table 2, entry C). Upon formation of triazole 5, generated without exclusion of moisture or air, the Cu/C was filtered (in air) and reused in at least two additional cycles without loss of activity (Table 2, entries D and E). In addition to unprocessed dioxane as the medium, other solvents appear to be of equal or even greater potential in these reactions. Toluene led to triazole 5 at room temperature at three times the rate seen in dioxane (entry F vs G, Table 2). Both 95 % EtOH (Table 2, entry H) and even pure water (entry I) are perfectly amenable to heterogeneous Cu/C-catalyzed click reactions, with these cycloadditions reaching completion at 60°C in less than 10 min.

To further examine the possibility of Cu/C catalysis in the absence of additives (e.g. Et₃N), reactions were exposed to controlled microwave irradiation (Table 3). As expected, the time needed for cycloaddition resulting in triazole 5 was reduced from 4 h to less than 10 min with the reaction temperature being increased from 60°C to 120°C. More remarkably, Cu/C quantitatively catalyzed formation of 5 in less than 3 min at 150°C with no loss of regioselectivity. This flawless 1,4-selectivity suggests that the process is discriminatory towards catalysis by copper (and not driven thermally), which is made all the more apparent when the same reaction in the absence of catalyst afforded no trace of triazole.

In addition to the simplicity and efficiency of cycloadditions carried out in the presence of Cu/C, product contamination by copper is minimized if not completely averted. Quantitative ICP-AES[15] analyses of triazole products from multiple reactions revealed insignificant copper; indeed, copper concentrations observed were too low for accurate measurement of these millimole-scale reactions. Thus, these data further suggest that Cu/C-catalyzed click cycloadditions take place heterogeneously with negligible leaching of copper into solution, a characteristic that could simplify both preparation and isolation of triazoles for many applications.

Most recently, we have further streamlined the preparation of Cu/C (see Experimental Section) by bypassing the distillation step. To demonstrate utility, a batch of catalyst (> 40 gram) was quickly prepared (Scheme 2) and tested

(no distillation or inert atmosphere)
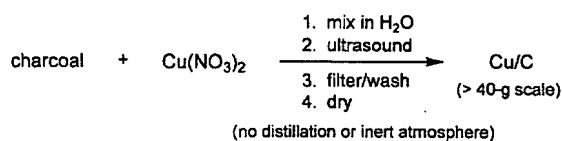
Scheme 2. Simplified, scaled-up preparation of Cu/C.

successfully in the conversion of 1 and 4 into 5. No noticeable difference in reaction rate or extent of leaching (by ICP-AES) was observed, notwithstanding impregnation of copper(II) nitrate.[16] Assuming catalysis by Cuᴵ, partial reduction by charcoal could account for the observed activity.

In conclusion, highly efficient click chemistry between organic azides and terminal alkynes can be heterogeneously catalyzed by copper nanoparticles mounted within the pores of activated charcoal.[9] Reactions can be accelerated with stoichiometric Et₃N or by simply increasing the reaction temperature. Under microwave irradiation, triazoles can be formed in minutes at 150°C. Cycloadditions can be carried out in a purely organic medium, in aqueous solvent mixtures, or in pure water. Solubility issues, copper contamination, and modest yields usually associated with the choice of copper salt are completely averted. External ligands known to accelerate click reactions are not needed. The catalyst appears to be

unaffected by exposure to air, suggesting a substantial shelf-life. Steric congestion in one or both partners is well-tolerated, and product isolation is notably facile.[17] Further studies that take advantage of Cu/C in several contexts (e.g. kinetic resolutions, mechanistic questions, formation of new triazole-containing ligands, etc.) are ongoing, the results from which will be reported in due course.

Experimental Section

Simplified preparation of Cu/C:[18] Darco KB activated carbon (50.0 g, 100 mesh, 25% H₂O content) was added to a 500-mL round-bottomed flask containing a stir bar. A solution of Cu-(NO₃)₂·3H₂O (Acros Organics, 11.114 g, 46.0 mmol) in deionized water (100 mL) was added to the flask, and further deionized H₂O (100 mL) was added to wash down the sides of the flask. The flask was loosely capped and stirred under air for 30 min and then submerged in an ultrasonic bath for 7 h. Subsequent washing (toluene) and air drying (3 h) by vacuum filtration yielded about 85 grams of "wet" Cu/C. The catalyst could be used at this stage or further dried in vacuo at 120 °C overnight (41 g yield of "dry" Cu/C).

General procedure for Cu/C-catalyzed "click" reaction: Cu/C (50 mg, 1.01 mmol g⁻¹, ca. 0.05 mmol) is added to a clean 10-mL flask fitted with a stir bar and septum. Dioxane (1–2 mL) is added slowly to the sidewalls of the flask to rinse the catalyst down. While the heterogeneous solution is stirred, triethylamine (1.1 mmol), alkyne (1.1 mmol), and azide (1.0 mmol) are added. The flask is stirred at room temperature (or warmed to 60 °C), and the reaction progress is monitored by TLC until complete consumption of azide has occurred. The mixture is filtered through a pad of celite to remove the catalyst, and the filter cake is further washed with EtOAc to ensure complete transfer. The volatiles are removed in vacuo to give pure triazole.

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[17] Removal of catalyst by filtration followed by evaporation of volatiles gives pure triazole products with little to no copper contamination. See Experimental Section.
[18] Some Cu(NO₃)₂·3H₂O remained in the filtrate and was recovered by evaporation of the water. Further optimization of this preparation is ongoing.

Conceptual, Qualitative, and Quantitative Theories of 1,3-Dipolar and Diels–Alder Cycloadditions Used in Synthesis

Daniel H. Ess,[a] Gavin O. Jones,[a] and K. N. Houk[a],*

[a] Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095-1569, USA
Fax: (+1)-310-206-1843; e-mail: houk@chem.ucla.edu

Abstract: The application and performance of conceptual and qualitative theories and quantitative quantum mechanical methods to the study of mechanism, reactivity, and selectivity of 1,3-dipolar and Diels–Alder cycloadditions are reviewed. This review emphasizes the application of conceptual density functional theory (DFT) for predicting reactivity and regioselectivity, and highly accurate quantum mechanical methods for predicting barrier heights and reaction energetics. Applications of computations to solvation effects, metal and organocatalysis, are also described.

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2   Qualitative Treatments
2.1 Frontier Molecular Orbital (FMO) Theory
2.2 Conceptual Density Functional Theory (DFT) and Hard and Soft Acid and Base (HSAB) Theory

2.3 Configuration Mixing Models
3   Quantitative Computation of Activation Barriers and Reaction Energetics
4   Solvent Effects and Catalysis
4.1 Aqueous Solvation Effects in Diels–Alder and 1,3-Dipolar Cycloaddition Reactions
4.2 Catalysis of Hetero-Diels–Alder Cycloadditions by Non-Aqueous Solvents
4.3 Hydrogen-Bonding Organocatalysis of Hetero-Diels–Alder Cycloadditions
4.3 Organocatalysis of Diels–Alder Reactions with Chiral Imidazolidinones
4.4 Catalysis of 1,3-Dipolar Cycloaddition Reactions
5   Conclusion

Keywords: conceptual density functional theory; configuration mixing; Diels–Alder cycloadditions; 1,3-dipolar cycloadditions; frontier molecular orbital theory; hard and soft acid and base theory

1 Introduction

Theory has played a significant role in the use of cycloadditions in synthesis.[1] Frontier molecular orbital (FMO) theory is a practical working tool for synthetic chemists and probably will be for some time. However, with the advent of density functional theory (DFT) and the ability to carry out experimentally accurate calculations, new conceptual models and quantitative quantum mechanical methods have been applied to a variety of 1,3-dipolar and Diels–Alder cycloadditions.[2] These recent qualitative and quantitative analyses have provided rich insights into reactivity, selectivity, and mechanisms of cycloadditions.

This article reviews the application and performance of conceptual theories and quantitative methods toward issues such as reactivity and selectivity.[3] This review is composed of three parts. Section 2 introduces FMO theory and its wide application, identifies evident shortcomings of this qualitative theory, and highlights popular conceptual and qualitative models for cycloadditions with emphasis on the emerging application of conceptual DFT for predicting reactivity and regioselectivity in Section 2.2. In Section 3, the most reliable quantitative thermochemistries for Diels–Alder and 1,3-dipolar cycloadditions are reviewed. Benchmark investigations of cycloadditions that provide critical evaluations of the accuracy and precision of *ab initio* and DFT methods are reviewed. Section 4 reviews recent applications of computations toward solvation effects, metal catalysis, and organocatalysis in cycloadditions.

2 Qualitative Treatments

2.1 Frontier Molecular Orbital (FMO) Theory

Frontier molecular orbital theory has proven to be a powerful conceptual framework to understand reactivities and selectivities in many types of reactions.[4] Developed by Fukui, it employs perturbation theory

Daniel Ess was born in 1981 in California and grew up in Utah. He attended a vocational college in his teenage years and then graduated from Brigham Young University in 2000. As a graduate student in K. N. Houk's lab, he is actively investigating cycloadditions using highly accurate quantum mechanical methods and developing new reactivity models. He is an IGERT-NSF fellow at UCLA.

Gavin Jones was born in St. Thomas, Jamaica in 1981. Since 2003 he has been a graduate student in the Department of Chemistry and Biochemistry at UCLA where he has been working in the labs of Prof. Kendall N. Houk's group at UCLA. His thesis work focuses on computational investigations of substituent effects on the rates of pericyclic reactions.

Kendall N. Houk obtained his Ph.D. in 1968 under the direction of R. B. Woodward. He has held faculty positions at Louisiana State University, University of Pittsburgh, and UCLA since 1985. He received the American Chemical Society James Flack Norris Award in Physical Organic Chemistry and the Award for Computers in Chemical and Pharmaceutical Research. He is a fellow of the American Academy of Arts and Sciences, the World Association of Theoretical and Computational Chemists, and the International Academy of Quantum Molecular Science. Among his major interests are the experimental and theoretical investigations of cycloaddition chemistry.

and emphasizes the interactions of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), the "frontier orbitals," of reacting molecules. Orbitals that are closest in energy interact most strongly. Smaller HOMO–LUMO gaps lead to lower activation barriers for reactions. Stabilization is also related to the square of the total overlap between the HOMO of one component and the LUMO of the other.

The prediction of reactivity, and regioselectivity patterns in pericyclic reactions is the most common application of FMO theory.[4a–f] The examples in Figure 1 show the normal electron demand Diels–Alder reaction between methyl acrylate and 1,3-dimethoxybuta-1,3-diene (Figure 1a and b) and the [2+2] cycloaddition of ethylene (Figure 1c), illustrate the principles of FMO theory which may be applied to other cycloadditions.[5]

For the reaction of dimethoxybutadiene with methyl acrylate, the signs of the lobes on the termini of the HOMO of the diene match those on the LUMO of the dienophile; the same is true for the combination involving the LUMO of the diene and the HOMO of the dienophile (Figure 1b). Both of these cycloadditions are allowed. In contrast, the cycloaddition of ethylene is forbidden because of the lack of stabilizing interaction between the LUMO and HOMO of the ethylene molecules (Figure 1c).

The interaction of the HOMO of 1,3-dimethoxybutadiene and the LUMO of the methyl acrylate has a gap of 11 eV; this separation, being smaller than that of the other combination provides the main stabilization of the transition state. Consequently, regioselectivity in pericyclic reactions is related to better overlap between the lobes of the orbitals that possess the largest orbital coefficients. In the FMOs of this example, the substituents polarize the frontier orbitals of the diene and the dienophile so that the β carbon of the LUMO of the dienophile and C-4 of the HOMO of the diene have the largest orbital coefficients. In the transition state, the degree of bond formation between these atoms is largest. The bond formed between C-4 and the β carbon occurs before the bond formed by C-1 of the diene and the α carbon of the dienophile. This leads to a degree of asymmetry of the transition state that is dependent on the ability of
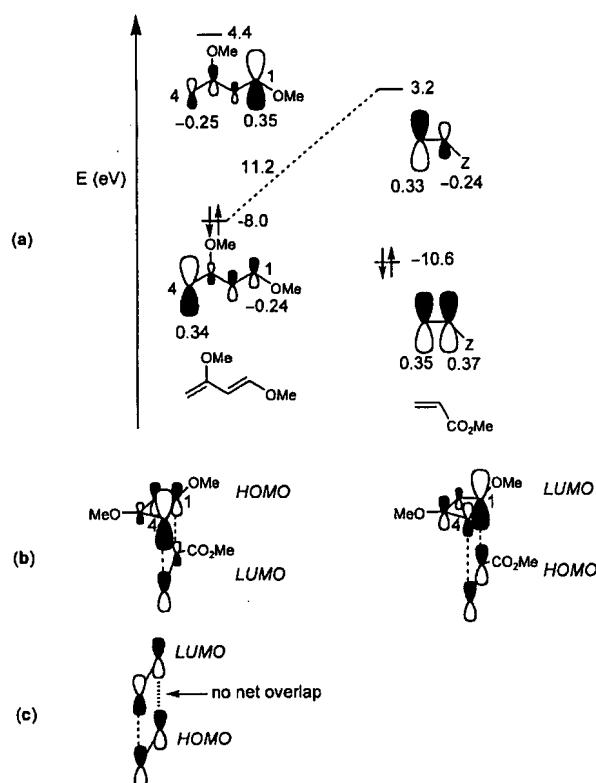
Figure 1. (a) Frontier molecular orbitals and (b) primary orbital interactions for the reaction of 1,3-dimethoxy-1,3-butadiene with methyl acrylate. Orbital energies by HF/6-31G(d) are shown in eV and orbital coefficients on reacting atoms are shown below the orbitals of the reactants. (c) Primary orbital interactions in the pericyclic reaction of two ethylene molecules.

the substituents on the diene and dienophile to stabilize the charge that develops on each reactant at the transition state.

For the vast majority of reactions considered, FMO theory predicts reactivities and selectivities that are in agreement with experiment. This, coupled with the ease of application of the method, has resulted in the widespread treatment of pericyclic reactions by FMO theory since being developed. Despite the popularity of FMO theory, however, a number of deficiencies have been identified, mainly due to the lack of quantitative significance of these FMO interactions. Alston and co-workers have pointed out that the interaction of secondary orbitals may at times outweigh the primary interactions that were shown in Figure 1.[6] These secondary orbital interactions involve orbitals that are not directly involved in the formation of new bonds, for example, the interaction of internal diene carbons with the electron-withdrawing substituent of the dienophile. Because the theory is only approximate, FMO theory does not always adequately ac-

count for the relative directing effects of substituents in polysubstituted dienes, or the influence of one type of substituent in different diene positions.[7] Singleton and co-workers have recently noted that because FMO theory utilizes ground state electronic configurations to describe reactivity, it may predict incorrect trends if the electronic configuration changes significantly near the transition state of the reaction.[8]

Recently, Spino et al. noted that in Diels–Alder cycloadditions involving electron-deficient dienes, FMO theory effectively predicts reactivity patterns of different dienophiles in normal electron demand reactions but failed to predict reactivities in inverse electron demand reactions.[9] In inverse electron demand reactions, unsymmetrical dienophiles are more reactive than symmetrical dienophiles. This is unaffected by the HOMO energies of dienophiles, and, therefore, the relative sizes of the $HOMO_{dienophile}$–$LUMO_{diene}$ gaps are not indicative of reactivities in these reactions. They suggest that the sizes of the orbital coefficients of the reactants are better indicators of the reactivity patterns in these types of reactions.

FMO theory remains a good qualitative guide to reactivity and selectivity, but because it is not a complete theory, it is not quantifiable.

2.2 Conceptual Density Functional Theory (DFT) and Hard and Soft Acid and Base (HSAB) Theory

The popularity and success of density functional theory (DFT) has stimulated many groups to use Hard and Soft Acid and Base (HSAB) theory, formulated with DFT, as a qualitative and quantitative treatment to predict reactivity based upon ground state properties (density) in a similar fashion to FMO theory.[10] This is an important development, since DFT is based upon the idea that electron densities can quantitatively account for energies and all properties; that is, molecular orbitals are unnecessary to explain chemistry. Conceptual DFT uses no orbitals, although it can be shown to be quantitatively related to FMO ideas. This theory has been applied to carbene reactivity,[11] S_N2 reactions,[12] enolate formation,[13] tautomerizations,[14] metal complexes,[15] enzymatic catalysis,[16] and many other organic reactions including cycloadditions.[17]

HSAB theory began as a classification of Lewis acids and bases as hard or soft based on properties such as ionization energies and polarizabilities.[18] Soft bases (electron donors) have high polarizability, low electronegativity, and low ionization energies. Hard bases have opposite properties, such as high negative charge, low polarizability and relatively high ionization energies. Soft acids (electron acceptors) have low charge, large size, are polarizable, and have high electron affinities. Hard acids are the opposite with large

positive charge, low polarizability, and low electron affinities. A basic axiom of HSAB theory is that soft-soft or hard-hard interactions are best. Soft-soft interactions are best described as charge transfer and arise from orbital interactions. Hard-hard interactions are mainly electrostatic in nature.[18c,d]

Over the last decade, Parr and others have laid a theoretical foundation of HSAB theory based on DFT principles.[2,10,19] The essence of HSAB, as formulated within DFT, is the transformation of the above definitions of hard and soft into chemical potential, μ, hardness, η, and softness, S. The interaction energy, ΔE_{int}, between two molecules is composed of two terms, a soft-soft (charge transfer or covalent) interaction term, ΔE_v, and a hard-hard (electron reorganization or electrostatic) interaction term, ΔE_μ.[20] These interaction terms are defined in terms of chemical potential, μ, and softness, S. The chemical potential μ, is the tendency of electrons to be transferred to or from a molecule to another molecule. It is defined as the partial derivative of the energy with respect to the charge, that is the change in energy of a system with the change in the number of electrons at a constant nuclear geometry. This partial derivative is often approximated as the ionization potential (IP) plus the electron affinity (EA) divided by $^1/_2$. When an IP is high (it is difficult to remove an electron), and the EA is high (easy to add an electron) the chemical potential, μ, is a large number. The negative of the chemical potential is the absolute electronegativity, χ. When two systems have different chemical potentials (different electronegativities), electron density flows from a high to low chemical potential (or from low electronegativity to high electronegativity) until equilibrium is reached and a new chemical potential is obtained for the composite system.[18]

The hardness value, η, (often expressed as its inverse, the softness value, S) defines how the chemical potential changes with change in electron number. The hardness is the second partial derivative of the chemical potential, and is often approximated to be (IP−EA)/2.[18] The difference between the IP and EA is related to the polarizability or hardness of a molecule. In orbital terms, this would be the HOMO–LUMO gap. A small HOMO–LUMO gap relates to high polarizability, resulting from mixing of filled with vacant orbitals and stabilization upon perturbation. Molecules with a large IP−EA or HOMO–LUMO gap, undergo little or no polarization upon perturbation. The molecule is hard, and resistant to change.

An electronegativity difference (or chemical potential difference) promotes the interaction of two systems by change or electron transfer, while the hardness resists the charge transfer interaction (Figure 2).[10,18,19] The ionization energy and electron affinity are properties of a molecule that are actually independent of orbitals, even though the connection
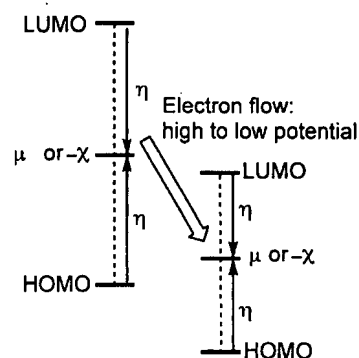
Figure 2. Qualitative diagram depicting the HOMO–LUMO gap, chemical potential, hardness, and electron flow between two species based on a difference in chemical potentials.

between HSAB theory and FMO theory is often made through Koopmans' theorem that equates the negatives of the HOMO and LUMO energies to the IP and EA, respectively.[10]

The chemical potential and hardness outlined above are molecular or global DFT descriptors. These provide an idea of reactivity. Application to the understanding of regioselectivity involves the Fukui function $f(r)$, which gives information equivalent to local softness.[19,21] The Fukui function, $f(r)$, is defined as the change in electron density at a given atomic site with the change in number of electrons at a constant nuclear geometry. An atomic site with a large Fukui function value is soft, while a site with a small Fukui function is hard.[20] Three Fukui functions exist that involve interactions with electrophiles or positive charge, $f^-(r)$, with nucleophiles or negative charge, $f^+(r)$, or a combination of the two as in radical or pericyclic reactions. The $f^-(r)$ and $f^+(r)$ are qualitatively related to the shapes of the HOMO and LUMO, respectively.[19e] From a practical standpoint, the values of these functions are approximated by computation of charges at each atom in the cation, anion, and neutral states:[2,10]

$f^-(r \approx q(N) - q(N-1) = q(Neutral) - q(Cation)$
electrophilic attack (acts as an nucleophile);
$f^+(r) \approx q(N+1) - q(N) = q(Anion) - q(Neutral)$
nucleophilic attack (acts as an electrophile);
$f^0(r) \approx (1/2)[q(N+1) - q(N-1)] = (1/2)[q(Anion) - q(Cation)]$
radical attack.

The condensed softness, s, which is often the DFT descriptor given for evaluating regioselectivity, is related to the Fukui functions by:[10]

$$s^- = S\, f^-$$
$$s^+ = S\, f^+$$

To evaluate regioselectivity, with similarities to matching FMO orbital coefficients, a local softness matching approach is effective at predicting the fa-

vored interaction orientation leading to the lower energy transition state. Essentially, predicting regioselectivity with softness matching corresponds to satisfying the HSAB theorem by matching the atomic sites with the closest softness values. Figure 3 shows the
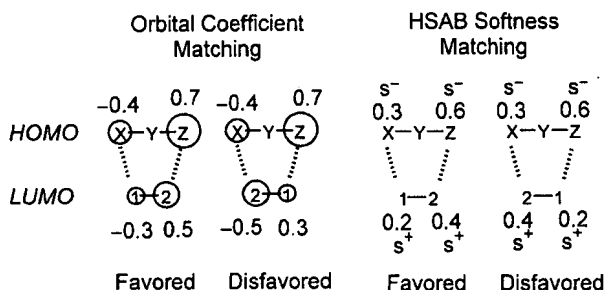

Figure 3. Hypothetical FMO orbital coefficient and HSAB softness matching schemes to evaluate regioselectivity.

corollary of softness matching with that of orbital coefficient matching for the hypothetical example of a nucleophilic 1,3-dipole interacting with an electrophilic alkene. As can be seen in Figure 3 the largest electrophilic attack softness value (meaning the most nucleophilic site), s^-, on the dipole is matched with the largest nucleophilic attack softness value (meaning the most electrophilic site), s^+, of the alkene in the favored interaction orientation. The alternative, disfavored orientation matches the largest values with the smallest values, and it is easy to see that this would not correspond to the softest interactions occurring to satisfy the HSAB principle. Whether to use the nucleophilic or electrophilic softness attack values for each of the reactants is based on the difference in chemical potential or evaluating electron transfer based on ionization potentials and electron affinities.

In the most representative example of applying softness matching and the HSAB principle, Chandra, Uchimaru, and Nguyen investigated the cycloadditions of hydrazoic acid and methyl azide with fluoro-, chloro-, methyl-, hydroxy-, and cyano-substituted ethylenes.[22] Using computed B3LYP ionization potentials and electron affinities of the neutral and charged dipoles and dipolarophiles, they showed that azides act primarily as electrophiles, except for the cycloadditions with cyanoethylene. Therefore, the local softness values for nucleophilic attack (s^+) for azide and methyl azide were matched with the local softness values for electrophilic attack (s^-) of the alkenes. For cyanoethylene, the opposite local softness attacks were considered. Scheme 1 gives the atomic softness values that were used to predict regioselectivity. As can be readily seen from the values in Scheme 1, for azide and methyl azide, the unsubstituted N terminal is the most electrophilic terminal with the largest nu-
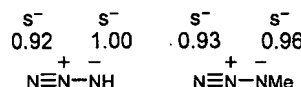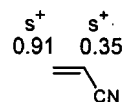
azides - nucleophilic attack values
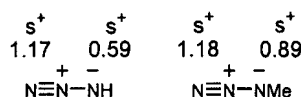
alkenes - electrophilic attack values
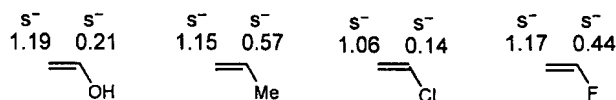
azides - electrophilic attack values
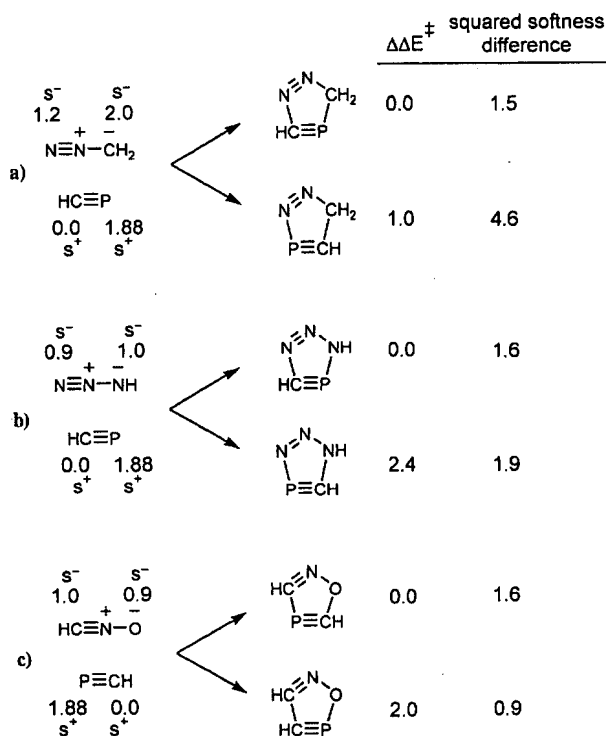

alkenes - nucleophilic attack values

Scheme 1. Atomic softness values for azide, methylazide, and substituted alkenes. Values taken from ref.[22]

cleophilic attack value (s^+), while the substituted terminal is the least electrophilic terminal. For the alkenes considered, the unsubstituted carbon is the most nucleophilic with the largest electrophilic attack (s^-) value. This means that the unsubstituted carbon has a greater ability to stabilize developing positive charge, and therefore the preferred regioisomers are those that unite the unsubstituted azide terminals with the unsubstituted alkene carbons. This also satisfies the HSAB principle, creating the softest possible interactions. For cyanoalkene, using the opposite nucleophilic and electrophilic attack values, shows a reversal in regioselectivity, where the substituted terminal of azide and methyl azide unite with the unsubstituted cyanoalkene carbon. The most nucleophilic substituted end of the azides unites with the unsubstituted end of cyanoalkene, which carbon can accommodate the developing negative charge.

Softness matching is commonly done using the sum of the squared difference in atomic softness values for the regioisomers. For the reactions considered in Scheme 1, the squared difference in softness values correspond to the regioselective preference compared to the computed B3LYP barriers.[22]

Nguyen et al. have also investigated the regioselectivity of the diazonium betaines of with halogen-substituted acetylenes and cyanides in a similar fashion.[23] In another study, Chandra and Nguyen, have investigated the reactivity and regioselectivity of di-

azomethane, hydrazoic acid, methyl azide, and ful-minic acid with HCP and $(H_3C)CP$ dipolarophiles.[24,25] They used the electrophilic attack values for the di-poles and nucleophilic attack values for the dipolaro-philes. Scheme 2 shows a few examples of their regio-

	$\Delta\Delta E^{\ddagger}$	squared softness difference
a) s^- s^- 1.2 2.0 $^+$ $^-$ N≡N—CH₂ / N≡N ... CH₂ HC≡P	0.0	1.5
HC≡P 0.0 1.88 s^+ s^+ / N≡N ... CH₂ P≡CH	1.0	4.6

b) s^- s^- 0.9 1.0 $^+$ $^-$ N≡N—NH / N≡N ... NH HC≡P	0.0	1.6
HC≡P 0.0 1.88 s^+ s^+ / N ... NH P≡CH	2.4	1.9

c) s^- s^- 1.0 0.9 $^+$ $^-$ HC≡N—O / HC ... N ... O P≡CH	0.0	1.6
P≡CH 1.88 0.0 s^+ s^+ / HC ... N ... O HC≡P	2.0	0.9

Scheme 2. Regioselective analysis for the cycloadditions of (a) diazomethane, (b) hydrazoic acid, and (c) fulminic acid with HCP. Electrophilic and nucleophilic softness attack values as well as the sum of the squared softness difference values and relative barrier heights in kcal mol⁻¹ (B3LYP/6-31G**).[24]

selectivity analysis for diazomethane, hydrazoic acid, and fulminic acid with HCP. Softness matching shows that N-C (O-C) and C-P interactions are preferred over N-P (O-P) and C-C interactions. The methylene terminal of diazomethane is more nucleophilic and can stabilize developing positive charge, while the phosphorus atom of HCP is very soft, and can easily stabilize the developing negative charge. The values of the squared difference in softness show numerically the preference in regioselectivity for this reaction as well as the reactions with hydrazoic acid and fulminic acid. The squared difference values magnify the slight difference in electrophilic attack value of hydrazoic acid and fulminic acid. This qualitative result is in agreement with the computed difference in barrier heights. However, the squared difference values do not quantitatively relate to $\Delta\Delta E^{\ddagger}$.

These reactions provide important examples of ap-plication of the HSAB principle to understand regio-selectivity; carbon and phosphorus atoms have similar FMO coefficients in both the HOMO and LUMO of HCP, and it is difficult to predict regioselectivity from an FMO approach.[24] Softness values are independent of frontier orbitals and correspond to the system as a whole, and have the potential to be more generally applicable than FMOs. The drawback is that no single generalization about which atoms are softer in cyclo-additions has been developed.

An example using softness matching for Diels–Alder reactions was reported by Damoun et al. They investigated the [4+2] reactions of terminally mono-substituted 1,3-butadienes with monosubstituted eth-ylenes (Scheme 3).[26] Reactions of 1-substituted

R = Me, OMe, CO₂H, NH₂, NMe₂, NEt₂, OEt, CN
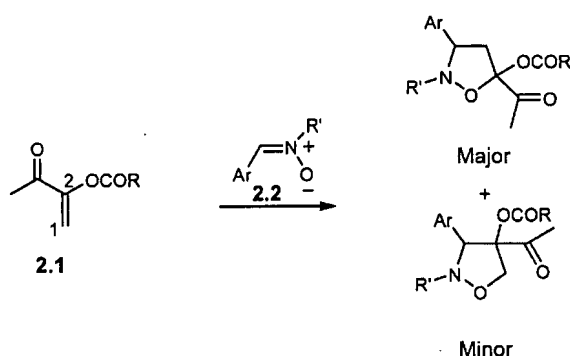
R' = CO₂H, CO₂Me, CN, CHO, NO₂, COMe
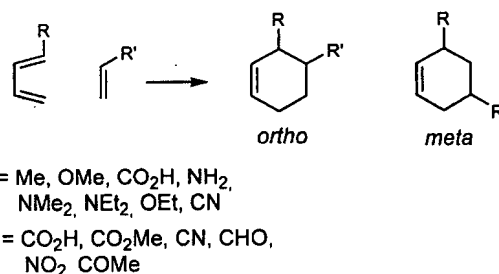
Scheme 3. The *ortho* and *meta* regioisomers of Diels–Alder cycloadditions of monosubstituted butadienes and ethylenes.

dienes with unsymmetrical dienophiles are of interest because replacement of an electron-withdrawing group with a donating group does not influence the regioselectivity.[27] Using the HF/3-21G method, they matched the local softness values for the *ortho* and *meta* regioisomers, and found that the vast majority of the 48 reactions had a smaller difference in softness for the *ortho* regioisomer than the *meta* one. This is in agreement with experimental results that give the *ortho* form as the major regioisomer.

Herrera et al. have investigated, experimentally and theoretically, the highly regio- and stereoselective 1,3-dipolar cycloaddition reactions of captodative 1-ace-tylvinyl carboxylates **2.1** with arylnitrones **2.2** and other 1,3-dipoles (Scheme 4).[28] They showed that re-gioselectivity is controlled because the electron-do-nating groups in the captodative olefins increase the interaction between the nitrone carbon atom and the β-C1 carbon of the α,β-unsaturated ketone. This subtle stereoelectronic effect was reflected in the elec-trophilic attack values of the nitrone carbon atom and the C1 atom of the captodative olefins. For the non-captodative methacrolein dipolarophile, this interac-tion is significantly lower because there is no longer a large nucleophilic tendency at C1. Therefore all regio-selectivity is lost.[29]
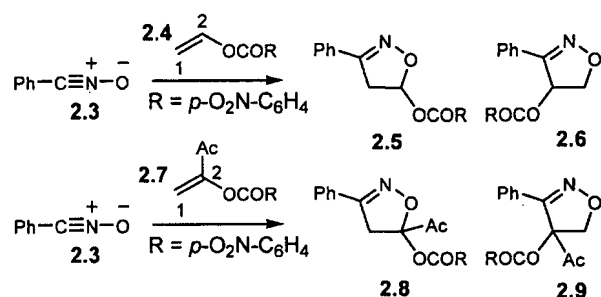
Scheme 4. Captodative 1,3-dipolar cycloadditions of 1-acetylvinyl carboxylates.

Scheme 5. Cycloaddition of benzonitrile oxide with vinyl p-nitrobenzoate and its acetyl derivative.

Chemical potential, hardness, and softness, can be used to calculate the interaction energy, ΔE_{int}, to compare the reactivities of dipolarophiles with 1,3-dipoles. These equations were first generalized by Parr and Gázquez and then extended for atomic interactions by Méndez and Gázquez.[19,20] The equation below is the approximate interaction energy between molecules A and B, where μ and S are the respective chemical potentials and softness values, and λ is a constant related to the number of valence electrons that are involved in the interaction.

$$\Delta E_{int} = (-1/2)\,\frac{(\mu_A - \mu_B)^2}{S_A + S_B}\,S_B S_A - (1/2)\,\frac{\lambda}{S_A - S_B}$$

Méndez, Tamariz, and Geerlings have used this equation for the reaction of benzonitrile oxide with vinyl p-nitrobenzoate and its acetyl derivative **2.7** that form isoxazolines **2.5** and **2.6** and **2.8** and **2.9**, respectively (Scheme 5).[30] Experimentally, the reaction of **2.3** with **2.7** gives exclusively the 5-acetyl-3-arylisoxazole **2.8**. Using the molecular values of μ and S to calculate ΔE_{int}, benzonitrile oxide has a 2.2 kcal mol^{-1} larger interaction energy with **2.7** than **2.4**. Using the local version of the ΔE_{int} for the possible regioisomeric interactions, the maximum interaction occurs when the C1 atom from **2.4/2.7** acts as the nucleophile towards the electrophilic carbon atom of benzonitrile oxide.

Ponti and Molateni have investigated 1,3-dipolar cycloadditions using a generalization of the HSAB principle in a semiquantitative manner by computing the grand potential, Ω, defined as, $E-N\mu$. E is the energy, N is the number of electrons, and μ is the chemical potential.[31] This grand potential is a measure of stabilizing interactions between two molecules. They used this approach to investigate cycloadditions

of C-carbomethoxy-N-(4-substituted)phenylnitrilimines **2.10**, aryl azides **2.11**, and (4-substituted)benzonitrile oxides **2.12** with methyl propiolate **2.13** shown in Scheme 6.[32–35]
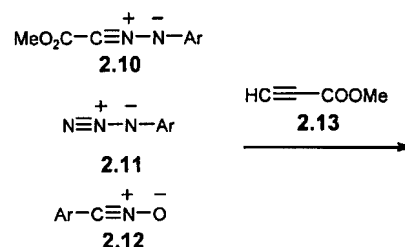
Scheme 6. 1,3-Dipolar cycloadditions with methyl propiolate.

The $\Delta\Omega$ values correctly predict the preferred regiochemistry preference for all reactions considered. The authors also showed that $\Delta\Delta\Omega$ is proportional to the barrier heights and this correlation can be used to estimate product ratios between the regioisomers. The strong correlation to the barrier height was attributed to the fact that $\Delta\Omega$ accounts for most of the energy difference between the regioisomeric transition states.

The following examples show the usefulness of another conceptual DFT descriptor called the electrophilicity index. First suggested by Maynard,[36] and then refined by Parr,[37] the so-called "electrophilicity index" ω, has been used as a measure for reactivity for a variety of reactions including 1,3-dipolar and Diels–Alder cycloadditions.[38] ω is the chemical potential squared divided by two times the hardness value.
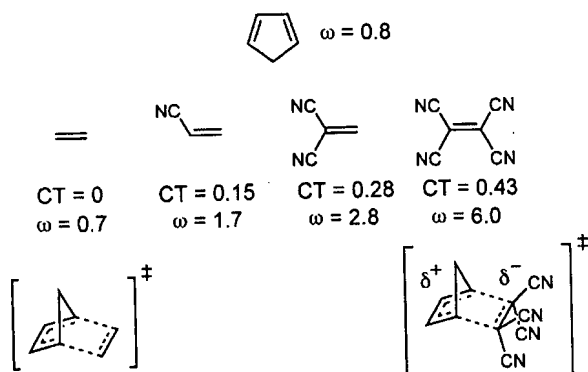
$$\omega = \mu^2/2\eta$$

Similar to the electron affinity, ω is a measure of the capability of a molecule to accept electrons. A large chemical potential, μ, reflects the tendency to acquire charge, and a small η value indicates a good electrophile.

In a series of papers, Domingo, Pérez and co-workers have investigated a variety of cycloaddition reactions using the global electrophilicity index. They characterized the most important 1,3-dipoles as strong, moderate, or marginal electrophiles according to their ω values.[39] Domingo and Contreras have also characterized a series of dienes and dienophiles commonly used in Diels–Alder cycloadditions.[40]

This index and classification have been promoted as a method to decipher the charge transfer direction between addends and predict concerted or asynchronous pathways based on the difference between ω values of a dipole/dipolarophile pair. The difference in the electrophilicity index Δω between dienes and dipolarophiles has been postulated to be an indicator of the polarity of a cycloaddition mechanism.

A representative application of the electrophilicity index was reported by Domingo and co-workers who investigated the polarity of Diels–Alder cycloadditions between cyclopentadiene and mono-, di-, tri-, and tetracyanoethylenes.[41] Scheme 7 shows the ω
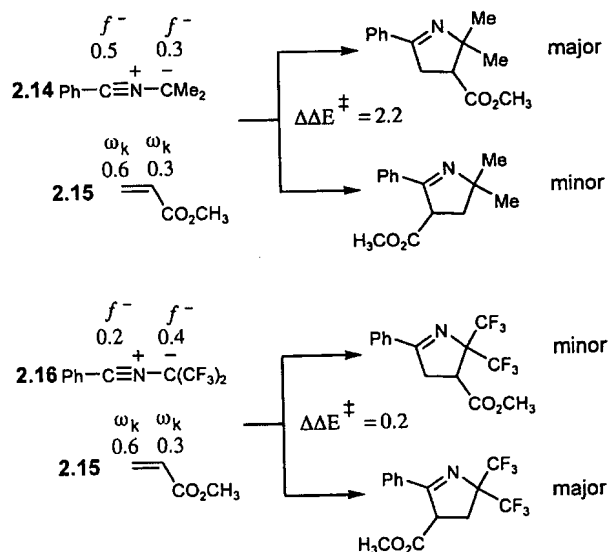
A global electrophilicity analysis was also carried out on a number of Lewis acid-catalyzed reactions showing that ω is a good predictor of Lewis acid polarization. Aluminum halide Lewis acid catalysts for reactions of nitroalkenes with vinyl ethers,[45] and boron heterocycle Lewis acid catalysts for the Diels–Alder reactions of cyclopentadiene and methylacrolein have also been investigated.[46] Domingo also applied this method to the Lewis acid-catalyzed reactions of N-acetyl-1-aza-1,3-buadiene with dimethylvinylamine,[47] and cyclopentadiene with arylidenoxazolone.[48] Ruano et al. showed that the increased reactivity of 5-ethoxy-3-p-tolylsulfinylfuran-2(5H)-one versus 5-methoxyfuran-2(5H)-one towards diazoalkanes could be rationalized based upon an electrophilicity index difference.[49]

In a different approach, Noorizadeh and Maihami have investigated the regioselectivity of 67 cycloaddition reactions using the electrophilicity index of the reaction products rather than the reactants. They found that the major product always had a lesser electrophilicity value; however, these results seem to be basis set dependent.[50]

The electrophilicity index, ω, also has a local equivalent, ω_k, which can be used to analyze regioselectivity.

$$\omega_k = \omega f_k^+$$

A number of studies have applied the combination of electrophilicity index model with local softness or Fukui function values to rationalize reactivity and regiochemistry.[51] Scheme 8 shows the values reported

Scheme 7. Charge transfer to ethylenes and electrophilicity index values for ethylenes for reactions of cyclopentadiene with ethylene, mono-, di-, and tetracyanoethylenes.[41]

values for cyclopentadiene and cyanoethylenes. Cyclopentadiene has a lower value of ω and shows that the direction of charge transfer is from diene to cyanoethylenes. The increasing ω values are in accord with the charge transfer to the alkene at the transition states. The electrophilicity values indicate the increasing polar character for these reactions and the large rate enhancements found experimentally.

A similar approach using an electrophilicity index was also applied to the polar Diels–Alder reactions of nitrosoalkenes with enamines,[42] and 1,3-butadienes with dimethyl acetylenedicarboxylate.[43] Domingo also showed that the low Δω values for the reactions of benzonitrile oxide with alkynylboronates explains the non-polar character of these transition states.[44]


Scheme 8. 1,3-Dipolar cycloadditions of nitrile ylides to methyl acrylate. Electrophilic attack Fukui functions on the 1,3-dipole terminals and atomic electrophilicity index, ω_k for the dipolarophiles are given, along with the B3LYP/6-31G* ΔΔE‡ values the regioisomers.[52]

by Aurell et al. for nitrile ylide **2.14** and **2.16** addition to methyl acrylate.[52] Methyl acrylate is considered a strong electrophile, and has a larger local electrophilicity value at the β-position, and is the preferred site of nucleophilic attack. For dimethylphenylnitrilium ylide **2.14**, the Fukui function for electrophilic attack shows that the dipole terminal that is phenyl-substituted is more nucleophilic. Matching these values leads to a correct prediction of the major regioisomer in accord with the computed difference in barriers. This example also highlights the sensitivity of the Fukui function to predict the reversal of polarization and regioselectivity for CF₃-substituted nitrile ylide **2.16** shown in Scheme 8. The CF₃ groups localize the negative charge and increase the nucleophilicity at that dipole terminal. This approach can only account for regioselective preference and does not necessarily correspond quantitatively to the difference in barrier heights.

Merino, Rescifina and co-workers have also rationalized the regiochemistry of *C*-(methoxycarbonyl)-*N*-methylnitrones with methyl acrylate and vinyl acetate,[53] and the nitrile oxide addition to anthracene and acridine using a similar approach.[54] Local electrophilicity has also been a successful criterion for rationalizing the regioselectivity for the Diels–Alder reaction of cyclopentadiene and 4-aza-6-nitrobenzofuroxan.[55]

In conclusion of this section, we note that Gayatri and Sastry have assessed the performance of DFT descriptors for 64 Diels–Alder reactions shown in Scheme 9 for predicting regioselectivity.[56] Using Fukui function matching from B3LYP calculations, 53 of 64 reactions could be rationalized by this approach. This highlights the potential broad applicability of DFT descriptors to predict reactivity and selectivity, but also the approximate nature of these descriptors.

X = N, P, CH
Y= NH, PH, O, S, CH₂
Z = NH, PH, O, S, CHMe, CHOH, CHCN, CHCHO
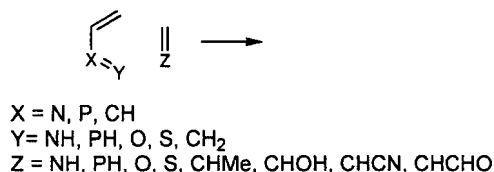
Scheme 9. Regioselective Diels–Alder cycloadditions assessed using FMO coefficients and DFT descriptors.

2.3 Configuration Mixing Models

The qualitative valence-bond configuration mixing model of Pross and Shaik describes reaction pathways based on mixing of ground and excited state configurations rather than orbital or density interactions.[57] The transition state, and reaction pathway, are functions of the configurations considered and the extent

to which they mix. This model, as implemented for cycloaddition reactions, focuses on the specific bonds that are broken and formed and their spin configurations. The barrier results from the crossing (or avoided crossing of states) of the reactants and product spin configuration wavefunctions. Figure 4 shows this
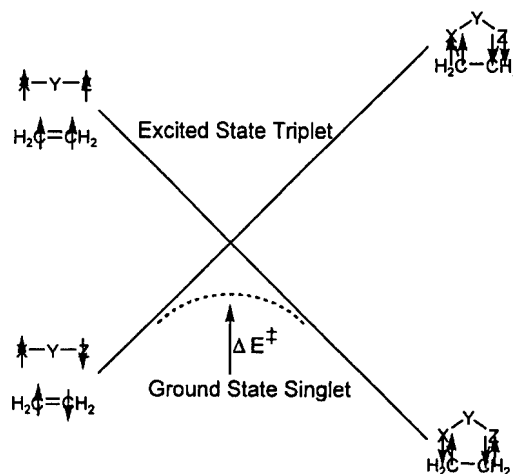
**Figure 4.** Configuration mixing model for 1,3-dipolar cycloadditions.

crossing and the resulting barrier for 1,3-dipolar cycloadditions with ethylene. Essentially, the singlet ground state of the 1,3-dipole and ethylene lead to the triplet excited state of the product, while the excited state triplet of the reactants leads to the singlet product ground state. Therefore, during the reaction, the intramolecular spin paired electrons in the 1,3-dipole and ethylene must decouple (transition from singlet to triplet spin states) to allow for the formation of the intermolecular spin paired σ-bond. Because the barrier height is controlled by the avoided curve crossing of the singlet and triplet states of the bonds that are formed and broken, it has been postulated that reactivity should correlate with the singlet-triplet energy gap (ΔE_{ST}) of the reactants. Therefore, the lower the ΔE_{ST}, the lower the barrier, the earlier the TS, and the greater is the exothermicity of the reaction energy. This is an elegant way of showing why increasing diradical character (which is related to decreasing the singlet-triplet gap) leads to high reactivity.

Su et al. investigated the reactions of nitrous oxide, hydrazoic acid, diazomethane, fulminic acid, formonitrile imine, and formonitrile ylide, the six prominent 1,3-dipoles "with a double bond", with ethylene using B3LYP and CCSD(T) methods.[58] They used CCSD(T) ground state singlet-triplet energy gaps (ΔE_{ST}) for these six 1,3-dipoles to rationalize barrier trends and reaction energies in a qualitative manner,

since the ΔE_{ST} value for ethylene is constant. The ΔE_{ST} (ΔE^* in parenthesis) values are 55.6 (28.7), 45.3 (13.2), 32.0 (12.8), 64.4 (11.2), 38.1 (6.3), and 27.2 (5.1) kcal mol^{-1}, respectively. A higher barrier and less exothermic reaction is associated with a larger ΔE_{ST} value for the diazonium and nitrilium betaine series, but there is not a linear correlation or general relationship.

In a related study, Liao et al. investigated the cycloaddition of nitrile ylide with cyano-, nitro-, chloro-, hydroxy-, and methyl-disubstituted ethylene derivatives.[59] They found that the lowest barriers were associated with highly electron-withdrawing groups. Using B3LYP, they observed that the smaller the ΔE_{ST} value, the lower the barrier. This general trend in reactivity is also observed in the ethylene HOMO–LUMO gaps. The lower barriers for electron-withdrawing groups were rationalized by the stabilization of the π^* orbital, which decreases the singlet-triplet splitting. This trend has also been rationalized by the favorable HOMO–LUMO interaction when the electron-rich nitrile ylide interacts with electron deficient dipolarophile.

Sakai and Nguyen have employed the so called "CAS-LMO-CI" method (complete active space-localized molecular orbital-configuration interaction) to investigate the contributing electronic configurations for the dipolar cycloadditions of diazomethane and fulminic acid with acetylene.[60,61] This method utilizes a full configuration interaction calculation on a localized (atomic) CASSCF/6-31G* wavefunction. The relative weights of localized molecular orbitals are analyzed along the minimum energy pathway (MEP). This type of treatment gives insight into the electronic reorganization along the reaction pathway, and the multi-reference wavefunction should give an accurate description of contributing configurations. Scheme 10 shows three possible electronic mechanisms possible for dipolar cycloadditions as defined by Sakai. I depicts a spin-coupled bond breaking and bond formation, a type of biradical coupling mechanism. II and III are "heterolytic" mechanisms, where the pairs of electrons migrate to form the new bonds, with II and III differing in direction of electron flow. This method provides a quantum mechanical version of the organic chemists' famous "arrow-pushing" formalism.

X = HC, Y = O (Fulminic Acid), X = CH₂, Y = N (Diazomethane)
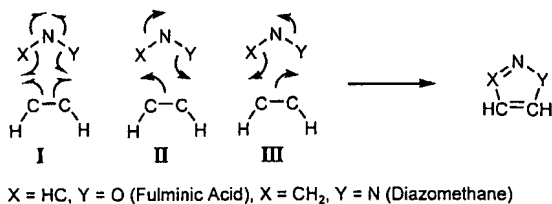
Scheme 10. Three electronic mechanisms for 1,3-dipolar cycloadditions.

When the CAS-LMO-CI method was applied to fulminic acid and diazomethane plus acetylene, two electron configurations had the largest change along the MEP (Figure 5). Configuration **A** decreased dra-

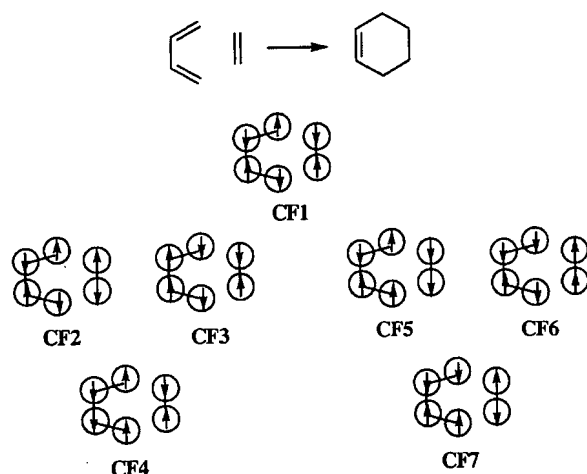X = HC, Y = O (Fulminic Acid)
X = CH₂ Y = N (Diazomethane)
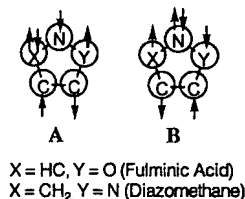
Figure 5. Configurations **A** and **B** describe the two major configurations at the transition state for fulminic acid and acetylene.

matically on going from reactants to products, while configuration **B** increased in weight along the minimum energy path. Based on this analysis, the authors concluded that the reaction mechanisms proceed through the coupling of biradical structures with slight charge polarization. For fulminic acid, the electron is initially transferred from the oxygen to the central nitrogen, and for diazomethane, electron movement occurs from the carbon atom to the central nitrogen.

The reactions of fulminic acid and diazomethane with NCH, HCP were also investigated. Depending on the nature of the dipolarophile, a diradical coupling or ionic type mechanism occurs. Ionic, heterolytic mechanisms were found for the reactions of HCNO and diazomethane with HCN were the new bonds had a large electronegative difference.[61]

Sakai has also used the CAS-LMO-CI method to investigate the stepwise and concerted pathways for the cycloaddition of 1,3-butadiene with ethylene.[62] The concerted reaction pathway is dominated by a single configuration **CF1** (Figure 6). In general, the largest weighted configurations for the reactants (**CF2–4**) correspond to π bonds in the Kekulé structure of butadiene and ethylene. These configurations decrease along the reaction pathway, while configurations **CF6–8** increase and correspond to the new σ bonds and π bond. Sakai points out that, at the transition state, configurations **CF2–7** are equally weighted, and this corresponds to its aromatic and concerted nature.

Karadakov et al. have used the spin-coupled CASSCF(6,6)/6-31G* method to investigate the electronic mechanisms for Diels–Alder and 1,3-dipolar cycloadditions.[63,64] For the cycloaddition of 1,3-butadiene with ethylene they found that a biradical coupling mechanism operates where three π bonds break and recouple to form two σ bonds and a π bond.[65] When this same method was employed to investigate

Figure 6. Most important configurations for 1,3-butadiene with ethylene and cyclohexene for the Diels–Alder cycloaddition.

the reaction of fulminic acid addition to acetylene, they found that three orbitals shift to create the two new bonds and the lone pair, which corresponds to the heterolytic mechanism II from Scheme 10. This is opposite to the predicted configurations by Sakai and co-workers. These studies have stimulated a mechanistic debate and culminated in two commentaries, and another investigation by Harcourt and Schulz. These authors advocate, opposed to the results of Karadakov et al., that fulminic acid and nitrous oxide have pentavalent nitrogens, and a valence bond consideration gives evidence for biradical coupling.[66,67]

Whatever the outcome of these investigations, there seems little doubt that the 1,3-dipolar and Diels–Alder cycloadditions have aromatic transition states that at least formally correspond to the union of two diradical wavefunctions.

Models to quantitatively define bonding in terms of σ-bonds, π-bonds, and lone pairs, typically conveyed in Lewis structures have also been developed and applied to cycloadditions. Polo et al. has used the electron localization function (ELF) method to investigate the mechanism of fulminic acid with acetylene,[68] while Berski et al. used ELF to investigate the reaction of 1,3-butadiene with ethylene.[69]

Weinhold's natural resonance theory (NRT) has also been applied by Mawhinney et al. towards understanding the electronic structure of nitrilimines.[70] They concluded that nitrilimines had significant carbene character. However, Ponti and co-workers have used CASSCF and spin-coupled calculations to show that the most important electronic structure of nitrilimines corresponds to the propargylic resonance form with little or no carbenic character.[71]
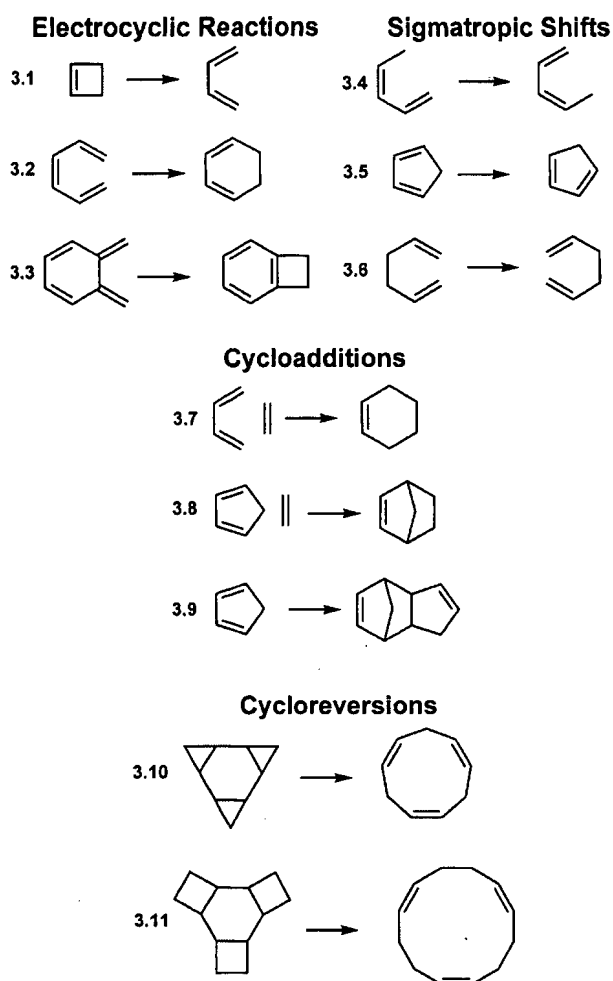
3 Quantitative Computation of Activation Barriers and Reaction Energetics

Only recently have experimentally accurate (defined as absolute error of $\pm 1\,\text{kcal mol}^{-1}$)[72] quantitative treatments of cycloadditions become possible by using compound quantum mechanical methods such as the G_n, CBS_x, or W_n series of methods.[73] These compound methods use successive quantum mechanical methods and empirical corrections to give the accurate thermochemistry values, albeit at a high computational cost. It is not feasible to use these methods on large systems of experimental interest. Density functional theory has become the attractive alternative because of its remarkable balance of speed and accuracy.[74] DFT has become the primary theoretical method for theoretical investigations of cycloadditions.[75] However, does DFT, especially the popular B3LYP, give experimentally accurate kinetic and thermodynamic energies? Recently, many investigations have been devoted to testing the performance of DFT methods for bond dissociation energies,[76] enthalpies of formation,[77] enthalpies of atomization,[78] proton affinities,[79] hydrogen abstraction reactions,[80] radical reactions,[81] weak interactions,[82] S_N2 reactions,[83] pericyclic reactions,[84,85] organic reactions,[86,87] metals,[88] materials,[89] biological applications, and even reactions relevant to astrophysics.[90]

This section reviews investigations that give the most reliable quantitative thermochemistries for Diels–Alder and 1,3-dipolar cycloadditions. Benchmark investigations of cycloadditions that give critical evaluations of the accuracy and precision of *ab initio* and DFT methods are also reviewed.

The quantitative prediction of activation barriers and reaction energies for Diels–Alder reactions in our laboratory has culminated with the proposal of a test set of hydrocarbon pericyclic reactions, including electrocyclic reactions, sigmatropic shifts, cycloadditions, and cycloreversions (Scheme 11), to be used as a standard to benchmark the performance of methods. Our lab has compared compound, *ab initio*, and DFT quantum mechanical methods to experimental activation barriers and reaction energies for this hydrocarbon test set.[91]

In this test set, reactions **3.7–3.9** are the cycloadditions of 1,3-butadiene with ethylene **3.7**, cyclopentadiene with ethylene **3.8**, and cyclopentadiene dimerization **3.9**. The experimental activation and reaction energies for **3.7–3.9**, corrected to 0 K using B3LYP thermochemical values, along with the values from the most accurate methods are listed in Table 1. Also listed is the mean absolute deviation (MAD) of activation (reaction) enthalpies for these methods for the entire hydrocarbon pericyclic test set, which give a good estimate of the accuracy.

Electrocyclic Reactions

3.1

3.2

3.3

Sigmatropic Shifts

3.4

3.5

3.6

Cycloadditions

3.7

3.8

3.9

Cycloreversions

3.10

3.11

Scheme 11. The standard set of hydrocarbon pericyclic reactions 3.1–3.11 used for benchmarking purposes.

The methods in Table 1 are listed in order of increasing MAD value for $\Delta H^{\ddagger}$ compared to experiment. The hybrid DFT methods, B3LYP, MPW1K,

and O3LYP along with the compound CBS-QB3, and CASPT2 all give MAD values under 2.5 kcal mol^{-1} and essentially statistically perform equivalently.[90,92] Linear correlations between experimental and computed $\Delta H^{\ddagger}_{rxn(0K)}$ values indicates that CBS-QB3 and CASPT2 methods gives the best agreement with experiment. B3LYP gives good agreement with experiment, but does not agree well with CBS-QB3 and CASPT2 for 3.8 and 3.9. For reaction energies, CBS-QB3 and CASPT2 show superior performance with MAD values of 1.6 kcal mol^{-1}, while DFT methods range from 3.9 to 6.2 kcal mol^{-1}. See ref.[93] for a brief description of these methods.

For the cycloaddition of 1,3-butadiene with ethylene, CASPT2 gives the exact $\Delta H^{\ddagger}$ value, and B3LYP only underestimates the barrier by 0.1 kcal mol^{-1}. In general, DFT methods have a small deviation from experiment (-0.6 to $+1.8$ kcal mol^{-1}) for $\Delta H^{\ddagger}$. For the reaction energy, only MPW1K deviates substantially. CBS-QB3 and CASPT2 underestimate the barriers (-6.4 and-5.1 kcal mol^{-1}) for reaction 3.8, while DFT methods deviate only slightly (-3.1 to $+0.2$). Only CBS-QB3 gives an accurate reaction energy with a deviation of-1.4 kcal mol^{-1}. The computed activation barriers for cyclopentadiene dimerization have a large range (12.7 to 24.1 kcal mol^{-1}), while experimental measurements have a range of 4.6 kcal mol^{-1}. The highly overestimated B3LYP value may be due to the systematic error for calculating norbornene structures and may be due to the inability of B3LYP to correctly predict the strain in norbornene structures or simply account for the proper electron correlation.[94] The CBS-QB3 and CASPT2 values of 11.6 and 12.7 kcal mol^{-1} may be a more accurate estimate of the barrier, and are close to gas phase measurements.

Morokuma and co-workers have also used high accuracy methods, CCSD(T), G2MS and G2MP2 to quantitatively investigate the barriers of butadiene with ethylene and acetylene 3.7a and b and cyclopentadiene with ethylene and acetylene 3.8a and b.[95]

Table 1. Experimental and computed $\Delta H^{\ddagger}_{rxn(0K)}$ and $\Delta H_{rxn(0K)}$ values for *ab initio*, DFT, and CBS-QB3 methods, along with the mean absolute deviation (MAD).

Reaction	3.7 $\Delta H^{\ddagger}$	ΔH_{rxn}	3.8 $\Delta H^{\ddagger}$	ΔH_{rxn}	3.9 $\Delta H^{\ddagger}$	ΔH_{rxn}	MAD $\Delta H^{\ddagger}$ (ΔH_{rxn})
Experimental[a]	25.0	−39.6	23.7	−23.2	15.9	−19.7	
B3LYP/6-31G*	24.9	−36.6	22.2	−18.6	21.1	−11.1	1.5 (4.1)
MPW1K/6-31+G**	24.4	−28.4	20.6	−30.3	19.2	−23.7	2.1 (6.2)
CBS-QB3	22.9	−38.3	17.3	−24.6	11.6	−22.2	2.3 (1.6)
O3LYP/6-31G*	26.8	−37.3	23.9	−19.7	24.1	−11.4	2.4 (3.9)
CASPT2/6-31G*//CASSCF	25.0	−39.7	18.6	−26.2	12.7	−24.2	2.4 (1.6)

[a] The experimental error ranges for reaction 3.7 is ±0.5 and ±1 kcal mol^{-1} for the activation and reaction energies respectively. For 3.8, the error range is ±1.6 kcal mol^{-1} for the activation energy. For cyclopentadiene dimerization, 3.9, see ref.[91] for a liberal discussion of the experimental error.
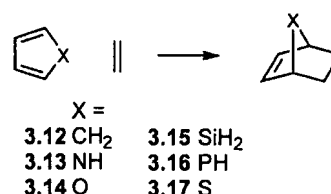
These values and the previously mentioned CBS-QB3 values are compared in Scheme 12. For the reaction of butadiene with ethylene, the G2 variants and CBS-

X =
3.12 CH₂   3.15 SiH₂
3.13 NH   3.16 PH
3.14 O   3.17 S

Scheme 13. Cyclic diene [4+2] reactions that were benchmarked by comparison to experimental and G3 values.

Scheme 12. Summary of highly accurate methods for the barriers of butadiene and cyclopentadiene with ethylene and acetylene.[90,91,94]

QB3 agree very well on a converged value of about 24 kcal mol⁻¹. For the other reactions shown in Scheme 12, the CCSD(T) values deviate from the G2MS and CBS-QB3 values. The G2MS and CBS-QB3 for cyclopentadiene and ethylene show a converged value of about 18 kcal mol⁻¹.

Sastry and co-workers investigated the reactions of 1,3-butadiene, cyclopentadiene, furan, thiophene, pyrrole, silole, and phosphole with ethylene using semi-empirical, *ab initio* and DFT methods (Scheme 13).[96]

They found that all methods were qualitatively able to reproduce barrier trends for these reactions, but Hartree–Fock and MP2 methods were quantitatively unreliable, while B3LYP and MP3 compared well to experiment and the CCSD(T) method.

Goumans et al. quantitatively accessed these same reactions with very accurate methods including the compound G3 method and QCISD(T).[97] The B3LYP, MP2, and spin-component-scaled MP2 (SCS-MP2)[98] methods were compared to these highly accurate methods. The most accurate methods, G3, CCSD(T), and QCISD(T) values are listed in Table 2 along with the mean average error (MAE) and maximum error for the B3LYP, MP2, and SCS-MP2 methods compared to the G3 values for activation and reaction energies combined. B3LYP and MP2 both perform somewhat poorly, while the SCS-MP2 method performs very well with a very large 6-311++G(3df,3pd) basis set.

A recent investigation by Jones et al. compared activation barriers computed by B3LYP, MPW1K and Hartree–Fock methods for the Diels–Alder reactions of cyclopentadiene and 9,10-dimethylanthracene with

Table 2. G3, CCSD(T), and QCISD(T) activation and reaction energies for cyclic dienes with ethylene, along with the mean average error (MAE) and maximum error for the B3LYP, MP2, and SCS-MP2 methods compared to the G3 values.

|		3.7	3.12	3.13[e]	3.14	3.15	3.16[f]	3.17
G3[a]	ΔE⁺	24.4	18.8	28.8	23.7	17.1	21.4	31.3
	ΔE_rxn	−37.8	−24.2	0.1	−10.2	−25.2	−19.9	−6.3
CCSD(T)[b]	ΔE⁺	25.1	19.8	28.3	24.7	19.8	23.6	32.7
	ΔE_rxn	−46.7	−31.0	−7.7	−16.1	−30.2	−26.4	−13.7
QCISD(T)[a]	ΔE⁺	27.4	21.9	30.6	26.7	21.5	25.3	34.5
	ΔE_rxn	−40.5	−25.2	−2.4	−11.1	−24.9	−21.0	−8.7
			MAE	Max. Error				
	B3LYP[c]		4.5	6.5				
	MP2[c]		5.4	9.2				
	SCS-MP2[d]		2.9	5.3				
			(1.1)	(2.4)				

[a] Values taken from ref.[96]
[b] Values taken from ref.[95], values do not include ZPE using the 6-31G* basis set. QCISD(T)/6-31G* at MP2 geometries, and include ZPE.
[c] 6-31G* basis set.
[d] 6-31G* and values in parenthesis are 6-311++G(3df,3pd).
[e] *anti*-transition state.
[f] *syn*-transition state.
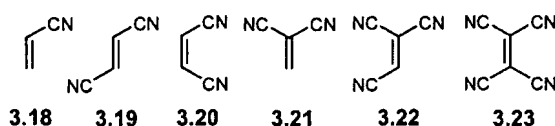
Scheme 14. Mono-, di-, tri-, and tetra-substituted ethylenes investigated for the Diels–Alder reactions with cyclopentadiene and 9,10-dimethylanthracene.

cyanoethylenes.[99] The cyanoethylenes considered in their investigation are shown in Scheme 14.

Hartree–Fock and MPW1K methods correctly predict the experimental activation enthalpy trends for the whole series, but B3LYP failed to predict the correct trends for **3.22** and **3.23**, with an increase in activation barrier for **3.22** and **3.23** compared to **3.21**, contrary to experiment (Table 3). These reactions have significant charge separation in the transition state, especially tetracyanoethylene, and methods with a larger fraction of exact exchange energy in the functional, such as MPW1K, perform best. For reaction enthalpies, HF and MPW1K perform well, while B3LYP underestimates the exothermicity.

Jones et al. also compared the activation barriers for 9,10-dimethylanthracene with $1,1,2,2-C_2(CN)_4$. MPW1K performs well with an activation enthalpy of $-1.2 \text{ kcal mol}^{-1}$ compared to $1.2 \text{ kcal mol}^{-1}$ measured experimentally. B3LYP performs very poorly, deviating by $11.5 \text{ kcal mol}^{-1}$ from experiment. These results are similar to those reported by Hehre and Domingo et al.[100]

For 1,3-dipolar cycloadditions, Ess and Houk quantitatively investigated the reactions of ethylene and acetylene with the nine 1,3-dipoles shown in Scheme 15 using the highly accurate CBS-QB3 method for the activation and reaction enthalpies.[101] Because there are no reported experimental measurements for dipolar cycloadditions of ethylene and acetylene with **3.24–3.32**, and very few experimental systems that can be treated with high accuracy methods, the CBS-QB3 values are taken to be the most accurate and the values were used as a standard to benchmark other methods.

The CBS-QB3 $\Delta H^+_{(0K)}$ and $\Delta H_{rxn(0K)}$ values are listed in Table 4 along with the average errors for B3LYP, MPW1K and MP2 methods compared to these CBS-QB3 values. The performance of the
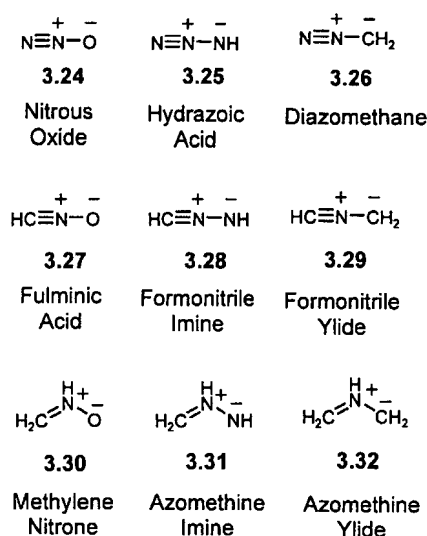
Scheme 15. Nine 1,3-dipoles from the three major classes.

B3LYP and MPW1K DFT methods was superb compared to CBS-QB3 for the computing of activation enthalpies as can be seen by MAD values of 1.5 and $1.1 \text{ kcal mol}^{-1}$. The reaction energetics were another story: B3LYP has a larger MAD, $2.4 \text{ kcal mol}^{-1}$, whereas MPW1K performed poorly with a MAD value of about 18 kcal mol^{-1}. The MP2 method gave the expected underestimation of barrier heights. All DFT methods performed more poorly with larger basis set.

Grimme et al. used these same CBS-QB3 values to evaluate the recently developed B2-PLYP DFT method and SCS-MP2 method.[102] Using the cc-pVTZ basis set, they found that SCS-MP2 performed very well for the activation energies of the 1,3-dipolar cycloadditions with a MAD value of $1.3 \text{ kcal mol}^{-1}$. This was a drastic improvement over the standard MP2 method with a $4.5 \text{ kcal mol}^{-1}$ MAD value with the same basis set. B2-PLYP performed better than B3LYP with 1.5 and $3.9 \text{ kcal mol}^{-1}$ MAD values respectively.

The reactions of diazonium and nitrilium betaines (**3.24–3.29**) with ethylene have also been treated with the CCSD(T) method by Su et al. (values given in Section 2.3). These values are close to the CBS-QB3 values. Two other studies have also applied the

Table 3. Activation barriers for the *endo*-Diels–Alder reactions of cyclopentadiene with cyanoethylenes.

Dieneophile		C_2H_4	3.18	3.19	3.20	3.21	3.22	3.23
Experimental	ΔH^+	22.5	15.5	12.8	12.8	9.0	7.6	5.0
B3LYP	ΔH^+	22.3	18.9	16.5	17.0	11.2	11.9	12.1
MPW1K	ΔH^+	20.7	16.3	12.9	13.4	8.9	7.7	5.6
HF	ΔH^+	42.0	37.8	34.4	34.5	30.0	28.0	26.1

Table 4. CBS-QB3 $\Delta H^{\ddagger}{}_{(0K)}$ and $\Delta H_{rxn(0K)}$ values for 1,3-dipolar cycloadditions of dipoles **3.24–3.32** with ethylene and acetylene, and the statistical comparison of DFT and *ab initio* methods against these values.

			3.24	3.25	3.26	3.27	3.28	3.29	3.30	3.31	3.32
CBS-QB3	$\Delta H^{\ddagger}$	ethylene	27.9	20.3	14.6	13.0	7.2	5.9	13.8	7.8	0.9
		acetylene	27.9	20.1	15.2	14.1	8.5	7.4	14.0	7.6	1.5
	ΔH_{rxn}	ethylene	−4.4	−19.7	−31.7	−39.3	−57.4	−68	−28.8	−44.2	−62.7
		acetylene	−37.1	−61.5	−49.0	−74.0	−100.3	−86.7	−43.9	−59.5	−76.9

		MAD	MD	Error Range
B3LYP[a]	$\Delta H^{\ddagger}$	1.5	0.3	6.6
	ΔH_{rxn}	2.4	−1.4	9.6
MPW1K[a]	$\Delta H^{\ddagger}$	1.1	0.3	4.1
	ΔH_{rxn}	17.9	−17.9	42.2
MP2[a]	$\Delta H^{\ddagger}$	3.4	−3.0	8.5

[a] 6-31G* basis set.

CCSD(T) method to the dipolar cycloaddition of diazomethane with ethylene. Blavins et al. computed a $\Delta H^{\ddagger}{}_{(0K)}$ value of 16.4 kcal mol⁻¹ with the CCSD(T)/6-311++G(2d,2p) method,[103] while Branchadell found the barrier to be 13.0 kcal mol⁻¹ using the CCSD(T)/6-311G** method.[104] The barrier for fulminic acid with acetylene was also investigated with CCSD(T) and G2 variants. All of the high accuracy methods give essentially the same value as the CBS-QB3 value within 0.5 kcal mol⁻¹.

4 Solvent Effects and Catalysis

4.1 Aqueous Solvation Effects in Diels–Alder and 1,3-Dipolar Cycloaddition Reactions

A fundamental principle of organic chemistry is that in comparison with less polar solvents, water may significantly accelerate or retard the rates of organic reactions involving charged species.[105] In contrast, the conventional expectation for Diels–Alder reactions involving hydrocarbon reactants is that the rate of the reaction is unaffected by solvent polarity.[106] This idea was reformulated after Breslow and co-workers discovered the surprising acceleration of neutral Diels–Alder reactions in aqueous solvents.[107] Currently accepted explanations of these results focus on acceleration of these reactions by both the hydrophobic effect,[107a] and by hydrogen bonding catalysis.[108] The hydrophobic effect accelerates the rate of the reaction by reducing the solvent accessible surface area (SASA) of the reactants during the cycloaddition process. In a normal electron demand Diels–Alder reaction, the LUMO energy of the dienophile with π-accepting groups is lowered by hydrogen bonding, leading to enhanced mixing with the HOMO of the diene. This interaction causes the transition state to be more polarized than the reactants, leading to enhanced hydrogen bonding of the solvent molecules with heteroatoms in the transition state. Preferential stabilization by this means lowers the activation energy for the reaction. Presumably, both factors are intertwined in most Diels–Alder reactions involving activated dienophiles that are accelerated by water.

Experimental[109] and theoretical[110] treatments have contributed numerous insights into the effects of aqueous solvation of Diels–Alder reactions. The results of the most recent study done by Jorgensen and co-workers lend support to earlier theoretical findings from his group and others.[111] A theoretical QM/MM approach was used to separate the contributions of enhanced hydrogen-bonding and the hydrophobic effect in Diels–Alder reactions of cyclopentadiene with acetonitrile, methyl vinyl ketone (MVK) and 1,4-naphthoquinone (Scheme 16). Monte Carlo simulations involving free energy perturbation calculations with the semi-empirical AM1 quantum mechanical method were used to generate free energy profiles for
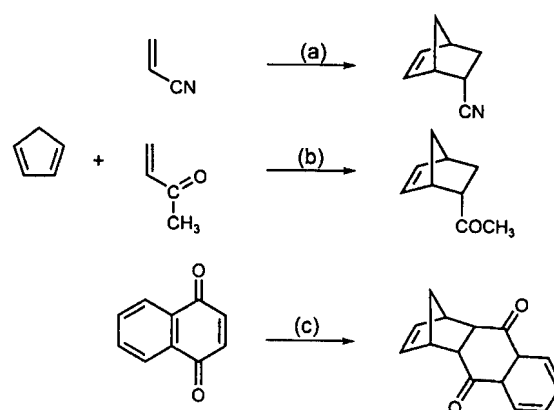
Scheme 16. Diels–Alder reactions of cyclopentadiene with (a) acetonitrile (b) methyl vinyl ketone (MVK) and (c) 1,4-naphthoquinone.

these reactions in the gas phase and in an aqueous environment of 500 water molecules.

Using this approach, the investigators found that in comparison with the gas phase, the activation energies for the reactions of cyclopentadiene with acrylonitrile, MVK and 1,4-naphthoquinone were reduced by 1.5, 2.8, and 4.4 $kcal\,mol^{-1}$, respectively, in water; these were in excellent agreement with experiment.

By evaluating the variation of the SASAs of these reactions along the reaction coordinate, the authors found that cycloaddition results in rapid reduction of the SASAs for all reactions, from the maximum value for the separated reactants, to a minimum at the transition states of these reactions. The hydrophobic effect contributes no more than 1 $kcal\,mol^{-1}$ to the reduction in the activation energies of the reactions, which implies that the acceleration of these reactions in water is mostly caused by differential stabilization of the transition states by hydrogen-bonding interactions with the solvent. Water molecules formed one more hydrogen bond on average with the transition states than with reactants, because the transition states had the greatest C^+–O^- polarization.

Reactions involving non-polar substrates should be influenced by the hydrophobic effect, while in polar cases like 1,3-dipolar cycloadditions, the substrates can form strong hydrogen bonds with the solvent. Therefore, 1,3-dipolar cycloaddition reactions would be expected to be accelerated to a lesser extent in water than corresponding Diels–Alder cycloadditions.

Despite these results, Butler et al. demonstrated experimentally that the rates of some 1,3-dipolar cycloadditions can be accelerated in pure water.[112] The cycloadditions of pyridazinium-dicyanomethides (Scheme 17) with alkenes are nearly unaffected by

Z = CHO, CRO (R = alkyl)
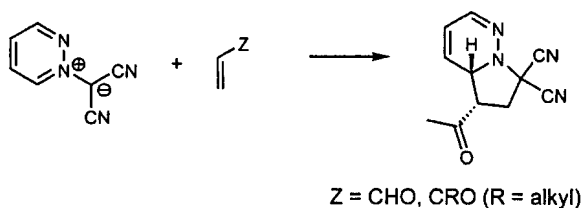
Scheme 17. 1,3-Dipolar cycloaddition reactions of pyridazinium-dicyanomethide with aldehyde and ketone substituted alkenes.

water if the alkene is substituted with ester, ether, sulfonyl, nitrile or aryl ring groups. However, the rates of reactions involving aldehyde or ketone-substituted alkenes are significantly accelerated in water, presumably because of enhanced hydrogen bonding at the transition state.

DFT calculations on the cycloadditions of pyridazinium-dicyanomethide with methyl vinyl ketone, show

that the activation enthalpies are reduced by 5–7 kcal mol^{-1} by water, whereas the activation enthalpies for reactions involving methyl acrylate are only reduced by about 1 $kcal\,mol^{-1}$.

4.2 Catalysis of Hetero-Diels–Alder Cycloadditions by Non-Aqueous Solvents

The aqueous acceleration of Diels–Alder cycloadditions has been a topic of interest since being discovered by Breslow and co-workers.[107] The related hetero-Diels–Alder cycloadditions of ketones with dienes are promoted by catalysis or by pressure,[113] but the effect of solvent on these types of cycloadditions was unknown until Rawal and Huang demonstrated that hetero-Diels–Alder cycloadditions of butadiene derivatives with simple unactivated ketones are accelerated by protic polar solvents but are relatively unaffected by polar aprotic solvents.[114] Inspired by these findings, Domingo and co-workers investigated hetero-Diels–Alder reactions with DFT methods to assess the role of protic solvents in the catalysis of the hetero-Diels–Alder cycloaddition between Rawal's diene and acetone (Scheme 18).[115,116]
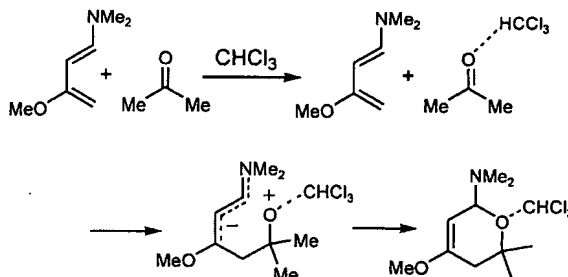
Scheme 18. Hetero-Diels–Alder cycloaddition reaction of N,N-dimethylamino-3-methoxy-buta-1,3-diene with acetone catalyzed by a molecule of chloroform solvent.

They found that inclusion of an explicit chloroform molecule hydrogen-bonding to the lone pairs of the carbonyl group of acetone reduces the activation enthalpy for the cycloaddition by 5 $kcal\,mol^{-1}$ in comparison to the uncatalyzed reaction, and changes the mechanism of the reaction from being concerted to being a stepwise mechanism involving zwitterionic intermediates (Scheme 18).[115]

They evaluated the nature of the hydrogen-bonding interaction with a combination of natural bond orbital (NBO) theory, atoms in molecules (AIM) theory and electron localization function (ELF), and energy decomposition analysis (EDA).[116] These demonstrated that the carbonyl group of the heterodienophile becomes more polarized in the transition state of the re-

action presenting one more lone pair for hydrogen-bonding with the solvent. Therefore, even weak hydrogen-bonding solvents are capable of catalyzing these reactions, however, only those solvents which form strong hydrogen bond interactions (for example, H_2O, CH_3OH and $CHCl_3$) are capable of exploiting this polarization.

4.3 Hydrogen-Bonding Organocatalysis of Hetero-Diels–Alder Cycloadditions

Metal-free catalysis of enantioselective organic reactions has generated great interest in recent years.[117] In particular, asymmetric induction in Diels–Alder reactions is one of the most challenging aspects of this field.[118] TADDOL catalysts developed by Rawal and co-workers catalyze Diels–Alder and hetero-Diels–Alder cycloadditions that are promoted by hydrogen-bonding interactions between diol groups on the catalyst and π-acceptors of the dienophile.[119] Zhang et al. reported experimental and computational studies on the catalysis and enantioselectivities of hetero-Diels–Alder reactions organocatalyzed by TADDOL derivatives (Scheme 19).[120]

They investigated these reactions with a computational model in which Danishefsky's diene was modeled with 1,3-dimethoxybuta-1,3-diene (Scheme 19). A layered ONIOM[121] approach was used in which the reacting moieties considered most important were modeled with a high-level method, while groups that were not deemed to be as important were modeled with a lower level method. This is represented in Figure 7, in which the substrates and the core of the catalyst were modeled with B3LYP/6-31G(d) and the backbone of the catalyst was modeled with PM3.

The computational study demonstrated that various diol catalysts provide moderate to excellent acceleration of the rates of these reactions, which are in good agreement with experiment. The TADDOL promoted cycloaddition is initiated by formation of an intramo-
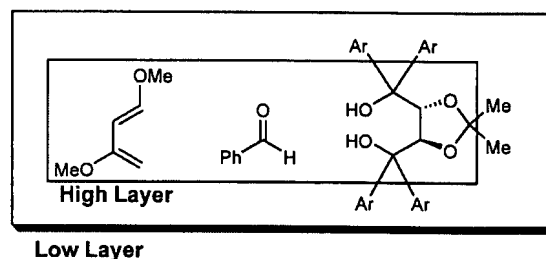
Figure 7. Layers used in ONIOM for geometry optimizations of the TADDOL-catalyzed cycloadditions of benzaldehyde and dimethoxybutadiene.

lecular hydrogen-bond between the hydroxy groups of the catalyst. This increases the Lewis acidity of the free hydrogen atom belonging to one of the free hydroxy groups, which forms an intermolecular hydrogen-bond with a lone pair of the carbonyl oxygen of the heterodienophile. The transition structures for the reaction between benzaldehyde and dimethoxybutadiene organocatalyzed by TADDOL **4.1** are shown in Figure 8.

The investigators account for the stereoselectivities observed in these reactions by invoking stronger

$$\Delta E^{\ddagger} = 10.0 \text{ kcal/mol} \qquad \Delta E^{\ddagger} = 12.6 \text{ kcal/mol}$$
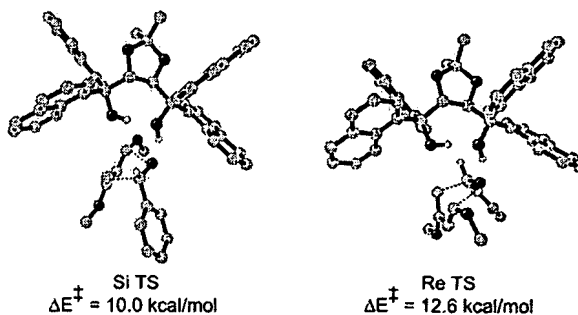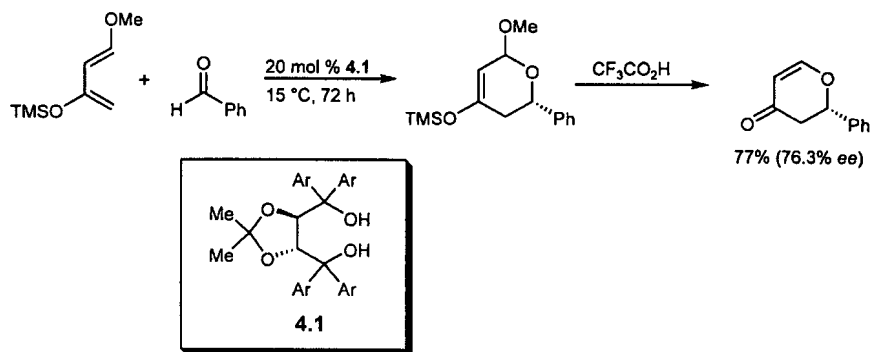
Si TS   Re TS

Figure 8. Transition structures for the hetero-Diels–Alder cycloaddition of benzaldehyde and 1,3-dimethoxybuta-1,3-diene catalyzed by TADDOL catalyst, **4.1**.


77% (76.3% ee)

Scheme 19. Hetero-Diels–Alder reaction between Danishefsky's diene and benzaldehyde organocatalyzed by the TADDOL catalyst, **4.1**.

steric repulsion between the aryl substituents on the diol catalysts and the phenyl group of benzaldehyde in disfavored transition states. Similar considerations also account for differences in stereoselectivity obtained with various TADDOL catalysts. Enantioselectivities are high for reactions in which the aryl groups on the catalyst backbone interact strongly with the substrate in disfavored transition states.

4.3 Organocatalysis of Diels–Alder Reactions with Chiral Imidazolidinones

MacMillan and co-workers have developed amine-based organocatalysts capable of catalyzing enantioselective Diels–Alder reactions,[122,123] as well as inducing asymmetry in other types of reactions.[124]

Houk and Gordillo[125,126] used B3LYP DFT to explore the amine catalyzed Diels–Alder cycloadditions of cyclopentadiene with a variety of α,β-unsaturated aldehydes and ketones (Scheme 20). Previous studies
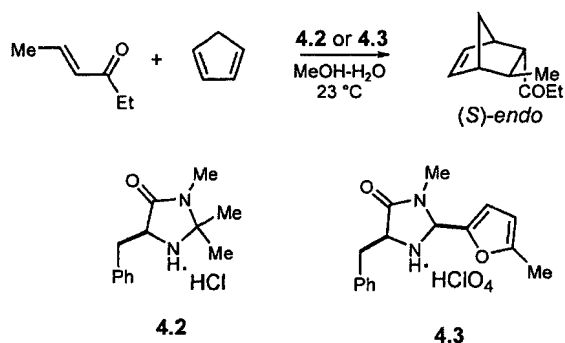
Scheme 20. Diels–Alder cycloadditions of cyclopentadiene with 4-hexen-3-one catalyzed by the chiral imidazolidinones 4.2 and 4.3.

out of the Houk group had demonstrated that DFT can be a powerful tool to predict enantioselectivities observed experimentally in a variety of asymmetric organocatalyzed reactions.[126,127]

Iminium ions formed from condensation of amines with α,β-unsaturated aldehydes and ketones reduce the activation barriers of Diels–Alder cycloaddition reactions in comparison with uncatalyzed reactions. B3LYP calculations account for the yields and selectivities observed in cycloadditions catalyzed by the chiral imidazolidinones. For example, the formation of the iminium cation from catalyst 4.2 is slow, or does not occur, because of steric encumbrance. As a result, the background uncatalyzed reaction occurs faster than the imidazolidinone-catalyzed reaction, leading to the low yield and non-selectivity observed experimentally. In contrast, the formation of the imi-

nium ion from catalyst 4.3 is fast, and there is a preference for attack in a sterically unencumbered fashion that leads to the high stereoselectivity observed for this reaction (Figure 9). Predicted selectivities are in excellent agreement with experiment.[125]
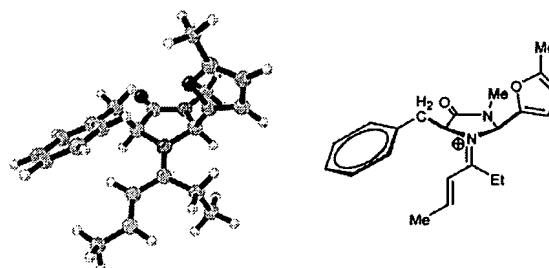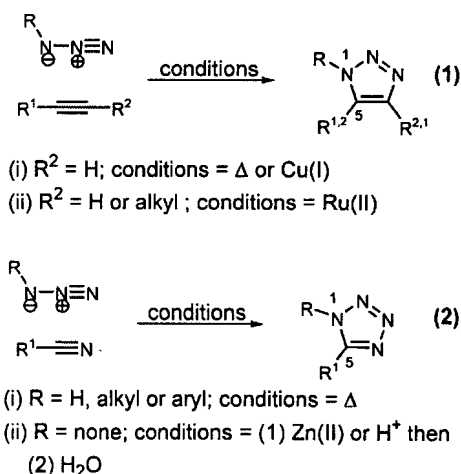
Figure 9. The iminium cation formed from condensation of chiral imidazolidinone catalyst 4.3 and 4-hexen-3-one.

4.4 Catalysis of 1,3-Dipolar Cycloaddition Reactions

The "click chemistry" methodology developed by the Sharpless group has been applied in a wide range of biological and materials chemistry contexts in the relatively short time since being introduced.[128] Arguably, the most appealing aspect of this strategy is the involvement of complementary substrates that are orthogonal to other functional groups and which participate in highly exothermic reactions.

1,3-Dipolar cycloaddition reactions of azides with terminal acetylenes (Scheme 21, 1) and nitriles (Scheme 21, 1 and 2), studied by Huisgen,[129,130] have been a focus of this research. The copper-catalyzed version, in which copper salts have been used to catalyze the reaction between azides and terminal acety-

(i) R^2 = H; conditions = Δ or Cu(I)

(ii) R^2 = H or alkyl ; conditions = Ru(II)

(i) R = H, alkyl or aryl; conditions = Δ

(ii) R = none; conditions = (1) Zn(II) or H^+ then
(2) H_2O

Scheme 21. Representative 1,3-dipolar cycloaddition reactions used in click chemistry.

lenes,[131] is the main reaction of click chemistry (Scheme 21, 1i). Recently, Sharpless et al. reported ruthenium-catalyzed cycloadditions between organic azides and monosubstituted, as well as disubstituted alkynes, that form 1,5-disubstituted 1,2,3-triazoles and 1,4,5-trisubstituted 1,2,3-triazoles, respectively (Scheme 21, 1ii).[132] Catalysis of reactions between organic nitriles and azide salts by magnesium,[133] zinc[134] and proton[135] (Scheme 21, 2ii) sources have also been studied.

While the mechanisms of the uncatalyzed versions of these reactions are well established,[136] the mechanisms by which adducts are formed by catalysis are less evident. The Scripps group, in an effort to elucidate the mechanisms of the uncatalyzed and catalyzed versions of these 1,3-dipolar cycloadditions, has relied on DFT methods to investigate the several possibilities involved in each of the acid-, zinc- and copper-catalyzed processes.

DFT calculations on the uncatalyzed reactions between nitriles and organic azides reveal that the reaction proceeds by a concerted [3+2] mechanism.[135] Calculations predict that the barriers for reactions of methyl azide with electron-deficient and electron-rich nitriles range from 18 kcal mol⁻¹ to 35 kcal mol⁻¹, respectively, and predict the preference for the formation of 1,5-disubstituted adducts over 2,5-disubstituted adducts, in excellent agreement with experiment.

Three mechanisms were considered for fast formation of 1-tetrazoles from reactions involving anionic azides and nitriles: the uncatalyzed process, and Lewis acid catalysis by proton sources,[135] and zinc salts.[137] Only mechanisms identified as being most probable are presented here.

The barriers for the uncatalyzed reactions of the azide anion with electron-rich nitriles are almost identical to the barriers for 1,3-dipolar cycloadditions involving neutral methyl azide. However, the activation barriers of reactions of the azide anion with electron-deficient nitriles are lowered by as much as 13 kcal mol⁻¹ in comparison with the organic azide. The most likely mechanism for acid catalyzed cycloaddition of azide anion to organic nitriles involves activation of the nitrile by proton sources proceeding from the eight-membered TS shown in Scheme 22, leading to the formation of intermediate P. Zinc catal-

ysis is similar, but is predicted to involve prior activation, by zinc, of a bound nitrile molecule, followed by cycloaddition with a free azide anion.[137]

Two possible mechanisms for the copper-catalyzed reactions of organic azides with terminal acetylenes[138] may involve either concerted addition of the azide to the η-complexed alkyne, or concerted addition of the azide to the copper-acetylide complex (Scheme 23a and b, respectively). DFT calculations reveal that the activation enthalpies for these concerted processes are comparable to activation enthalpies for the uncatalyzed, concerted variant. Alternatively, the mechanism may involve stepwise addition of the azide to the copper-acetylide complex followed by attack of the distal nitrogen to C2 of the acetylide resulting in the formation of a 6-membered metallacycle (Scheme 23c). Ring contraction results in the exothermic formation of the 5-membered triazolyl copper complex, that affords the 1,2,3 triazole after protonation. The activation enthalpy for the rate-determining step of this process, formation of the 6-membered metallacycle, is 11 kcal mol⁻¹ lower than the barrier for the uncatalyzed cycloaddition, corresponding to a 10⁵ acceleration in the rate of the reaction.

The stepwise mechanism adequately accounts for formation of 1,4-disubstituted adducts from the azide. Furthermore, investigations demonstrate that copper-catalyzed cycloadditions of acetylenes with other dipolarophiles, such as isoxazoles, may also involve this stepwise process.[138]

The ruthenium-catalyzed reactions of azides with disubstituted alkynes (Scheme 21, 1ii),[132] illustrates that catalysis of 1,3-dipolar cycloadditions may not only involve coordination of Lewis acids to the nitrogen atom of the nitrile, or formation of metal-acetylide complexes from terminal acetylenes as general mechanisms. Evidently, while copper-acetylides are preformed from terminal alkynes, formation of metallo-alkyne η-complexes may be the preferred mechanism in ruthenium catalyzed cycloadditions. These findings expand the scope of reactants and catalysts that may be used in these 1,3-dipolar cycloaddition reactions, and suggest new frontiers in the catalysis of 1,3-dipolar cycloadditions.

In connection with the results from the Scripps group, Kuznetsov et al. reported computational inves-
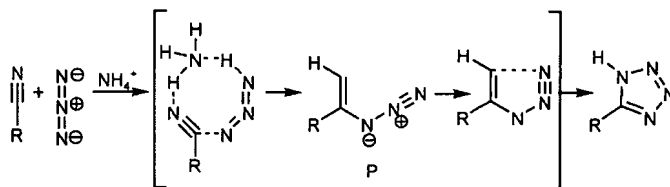

Scheme 22. Mechanism for formation of 5-tetrazoles from anionic azides and nitriles catalyzed by the ammonium cation, as predicted by B3LYP.
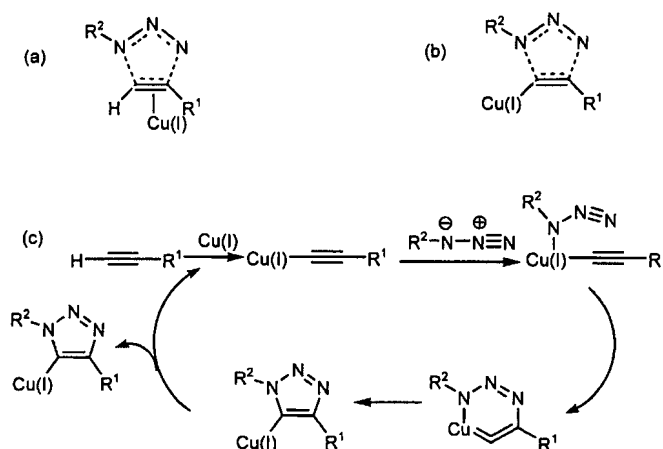
Scheme 23. Possible mechanisms in the copper-catalyzed 1,3-dipolar cycloadditions of organic azides and terminal acetylenes.

tigations on reactant activation in Pt(II)- and Pt(IV)-catalyzed cycloadditions of cyclic nitrones with nitriles to form oxadiazoline cycloadducts (Scheme 24). The
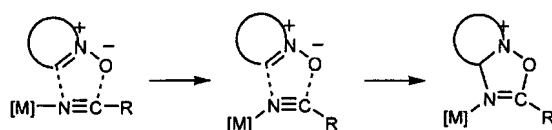
[M] = Pt(II) or Pt(IV)

Scheme 24. Platinum-catalyzed 1,3-dipolar cycloadditions of activated nitrones with organic nitriles.

investigators found that activation enthalpies for cycloadditions of Pt-bound nitriles with nitrones were considerably reduced in comparison with the corresponding uncatalyzed reactions.[139]

5 Conclusion

This review has discussed the application and performance of conceptual theories and quantitative quantum mechanical methods to the study of mechanism, reactivity, and selectivity of 1,3-dipolar and Diels–Alder cycloadditions. Conceptual DFT can be effective at understanding the nucleophilicity and electrophilicity of reactants and atomic sites of molecules, and when the HSAB principle is applied, leads to prediction of regiochemistry. The applications of configuration mixing models in cycloadditions have been discussed. The applications of recently developed highly accurate quantum mechanical methods such as CBS-QB3, G3, G2MS, G2MP2 all give essentially the same values for barrier heights and reaction energetics for

cycloadditions. DFT and *ab initio* methods vary in their ability accurately predict barriers and reaction energetics, depending on the test set used. Applications of computations to the study of solvation effects, organocatalysis and metal catalysis on the rates of 1,3-dipolar and Diels–Alder cycloadditions conclude the review.

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Study of high glass transition temperature thermosets made from the copper(I)-catalyzed azide–alkyne cycloaddition reaction

Nicolas Le Baut [a], David D. Díaz [b], Sreenivas Punna [b], M.G. Finn [b], Hugh R. Brown [a],*

[a] *Faculty of Engineering, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia*
[b] *The Scripps Research Institute, La Jolla, CA 92037, USA*

Abstract

Thermal properties of polymers made from the copper(I)-catalyzed cycloaddition reaction between azides and alkynes have been investigated. Differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA) were used to measure the glass transition temperatures (T_g) of these materials. The polymers were found to have unusually high T_g values, up to 60 °C higher than the curing temperature. It has been shown that the increase of T_g was time dependant, thus, depending on the state of cure of the material.
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Keywords: Glass transition temperature; Click chemistry; Copper

1. Introduction

Sharpless and colleagues published in 2001 an analysis of the most efficient chemical reactions that can be used to stitch organic fragments together to make complex functional molecules [1]. The authors argued that biological activity — the function of greatest concern to the organic synthesis community — should be easier to achieve by restricting synthetic operations as much as possible to such reactions, grouped under the name "click chemistry". While something of a controversial proposition in organic synthesis and medicinal chemistry, the use of a limited number of supremely reliable bond-forming methods to achieve sophisticated function is of course the foundation of polymer science. We seek to introduce into polymer chemistry some of the click reactions that have been explored thus far only in the context of drug discovery to broaden the base of connections available to make functional materials.

The copper(I)-catalyzed cycloaddition reaction between organic azides and terminal alkynes [2,3] to make 1,4-disubstituted 1,2,3-triazoles has emerged as the most versatile click

reaction for making connections to biological molecules [4–8] and for the discovery of biologically-active compounds [9–11]. The reaction is useful in biological settings for two main reasons: the azide and alkyne components are largely unreactive with biological molecules (and therefore selectively reactive with each other), and the product triazole can interact with biological structures in several noncovalent ways while being at the same time extraordinarily stable. The azide–alkyne cycloaddition has also rapidly captured the attention of investigators in materials science for similar reasons [12–21]. The synthesis of the linear polymer 3 is illustrative of a condensation polymerisation involving the azide–alkyne cycloaddition (AAC) reaction, as shown in Scheme 1. We have previously reported that multivalent azides and alkynes, when deposited between surfaces containing metallic copper, are crosslinked by Cu(I)-mediated triazole formation to give strong adhesion of one surface to the other [22]. Here we report the results of follow-up studies on two of these systems as well as the properties of the analogous polymers made in bulk. An unusual relationship between glass transition temperature and curing temperature has been established.

1,2,4-Triazoles are widely used in corrosion inhibitors and adhesion promoters on copper or copper based products

* Corresponding author. Tel.: +61 2 4221 3820; fax: +61 2 4221 3662.
  E-mail address: hugh_brown@uow.edu.au (H.R. Brown).
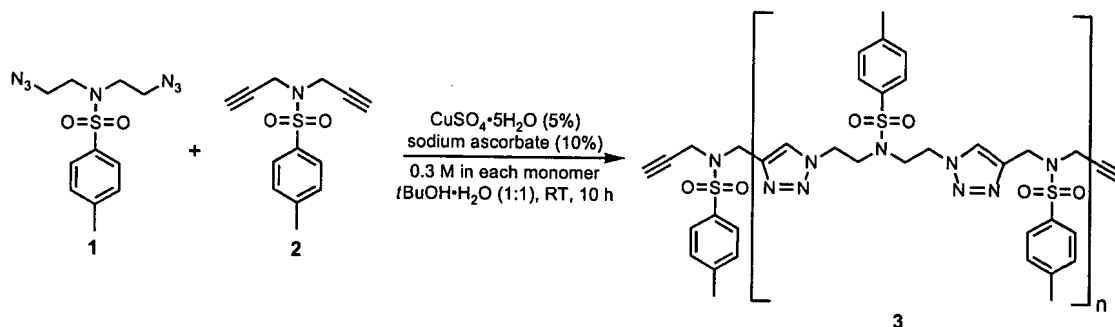
Scheme 1. Synthesis of a main-chain triazole polymer using the azide–alkyne cycloaddition reaction.

[23–29]. The poor durability of copper in oxidative environments derives from the inability of copper oxide to protect the surface toward continued corrosion. Polymers incorporating 1,2,4-triazoles act as a stabilizing inert film by covering the vulnerable oxide layer [28]. With the exception of benzotriazoles, the 1,2,3-triazole isomer is poorly represented in the adhesives and coatings literature because of the difficulty of its synthesis prior to the development of the Cu(I) catalytic procedure. However, our work provides evidence that 1,2,3-triazoles have good affinity for metallic surfaces and are far more stable than 1,2,4-triazoles to attack by other chemical entities. We therefore believe that 1,2,3-triazole-based materials have advantageous properties for high-performance metal coatings and adhesives. Since Cu(0)-containing surfaces can supply Cu(I) ions (presumably by comproportionation with Cu(II) derived from air oxidation), the azide–alkyne cycloaddition reaction is "self-starting" on such surfaces (Scheme 1).

2. Experimental

2.1. Polytriazole adhesives: bulk polymerisation

Quantities of 1 and 4 (3:2 molar ratio) or 5 and 6 (2:1 molar ratio), each providing an equivalent concentration of azide and alkyne groups, were stirred in THF (tetrahydrofuran, 99.9%, high-performance liquid chromatography (HPLC) grade, inhibitor-free, from Sigma–Aldrich) for at least 30 min until the solution appeared to be homogeneous. Copper(I) iodide (99.999% from Aldrich) was added as a fine powder (10% in weight) to the mixture over 2 min with vigorous stirring. The soluble Cu(I) complex [Cu(CH$_3$CN)$_4$]PF$_6$ was used as catalyst (10% in weight) for reactions of 5 and 6. The THF solution was immediately spread with a Pasteur pipette on the surface of the brass plates (cleaned just before use with acetone and

toluene). Before sticking the plates together, the solvent was allowed to evaporate for between 10 s and 3 min.

Samples prepared between copper or brass plates were cured at three different temperatures (room temperature, 50 °C, and 100 °C) and were tested after curing times varying from 2 to 30 days. Samples for differential scanning calorimetry (DSC) measurements were obtained by prying apart the glued plates and scraping the sample off the copper surface just prior to analysis.

2.2. Analytical methods

Modulated differential scanning calorimetry (MDSC) was performed with a DSC Q100 instrument (TA Instruments, Inc.) in modulated mode to separate the reversing heat flow (characterizing the glass transition temperature, T_g) and the non-reversing heat flow. Non-hermetic aluminium pans were used. The temperature was swept from 0 °C to 250 °C at a rate of 3 °C/min and a modulation period of 60 s. T_g values were determined using the midpoint method integrated in the software (Universal Analysis).

Dynamic mechanical analysis (DMA) was performed on samples made by joining two rectangular thin brass shims (35 mm long, 10 mm wide, 0.06 mm thick) with the polymer in between. The thickness of the adhesive between the shims varied from 0.07 mm to 0.27 mm. Measurements were performed with a DMA Q800 instrument (TA Instruments) using single cantilever clamps. Samples were held horizontal by two clamps with a sample length of 17.5 mm between them. One clamp was fixed, whilst the other applied a vertical displacement the sample. Samples were subjected to a temperature ramp from −20 °C to 200 °C at a rate of 3 °C/min. The clamp displacement was 50 μm with a frequency of 10 Hz with a maximum strain in the sample of 1.21×10^{-4}. The glass transition temperature was calculated as the mean of the loss modulus peak and the tan δ peak.

3. Results

3.1. Thermal properties of mixture 1 + 4

The mixture of diazide 1 and trialkyne 4 was chosen as a representative of an adhesive of average strength, as
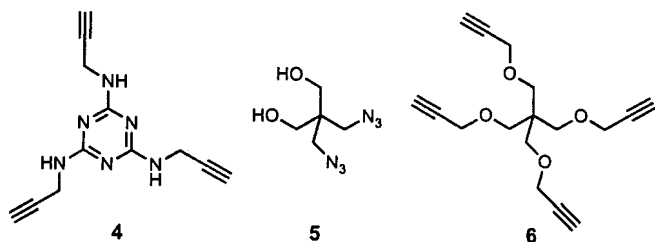
Fig. 1. Azide and alkyne compounds used in the reactions.

determined in our preliminary measurements [22]. The cross-linked polymer was prepared by polymerisation in solution as well as by bulk (solvent free) polymerisation between copper-containing plates. MDSC measurements on the solution prepared material showed an endothermic peak on the non-reversing heat flow curve at approximately 50–70 °C (Fig. 2). Similar results were obtained on the bulk polymerised material. When a sample of bulk polymerised material was crushed into powder and then heated at 200 °C for 24 h, MDSC data recorded immediately thereafter showed no such endothermic peak. Analysis every few days of a sample stored at room temperature in air then showed the endothermic peak growing in intensity as a function of time (Fig. 3). The T_g of the material (as measured by MDSC) was also found to decrease in a similar time-dependant fashion. The endothermic peak is therefore probably due to the evaporation of water that had absorbed into the hygroscopic material, with the residual (that remaining at temperatures above 180 °C) water acting as a plasticizer in its effect on the glass transition.

Preparation of an adhesive between copper plates was usually performed using THF solutions of the monomers. Evaporation of the solvent was monitored by the measurement

of weight under the typical conditions of curing between brass cantilever plates at room temperature (Fig. 4). A rapid loss of solvent was noted even though the adhesive mixture was exposed to air only at the plate edges. Approximately 25% of the THF was lost in the first hour and 94% evaporated after 3 days. A final value of 97% (leaving 3% solvent remaining) was achieved after 1 week. Hence the polymerisation process that occurs over several days may be considered to be a process in the bulk rather than in solution.

MDSC analysis of samples made between copper plates without additional catalyst shows that curing was quite slow compared to the reaction in solution, as expected (Fig. 5). The T_g value of the material made from 1 + 4 attained a value of up to 60 °C above the curing temperature after 10–15 days, and this behaviour of a T_g well above the curing temperature was observed for three curing temperatures (RT, 50 °C, and 100 °C). The results of DMA analysis of the adhesive formed by spreading the same mixture of monomers (1 + 4) between brass foils is shown in Fig. 6. T_g values determined in this manner followed the same trend as those measured by MDSC. Judging by these T_g profiles, the polymer reached a curing level of approximately 90% after 4 days when
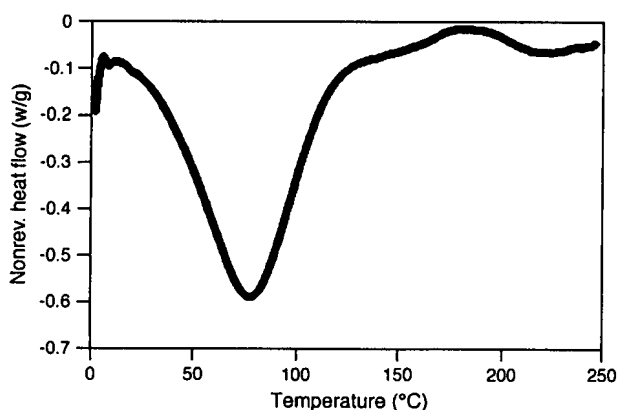
Fig. 2. MDSC analysis of the polymer prepared from 1 + 4, prepared in solution at 60 °C. The non-reversing heat flow shows an endothermic peak at 70 °C.

Fig. 4. Solvent evaporation as a function of curing time for a typical cantilever adhesive.

Fig. 3. Evolution of the MDSC endothermic peak size and T_g with the time of exposure to air.
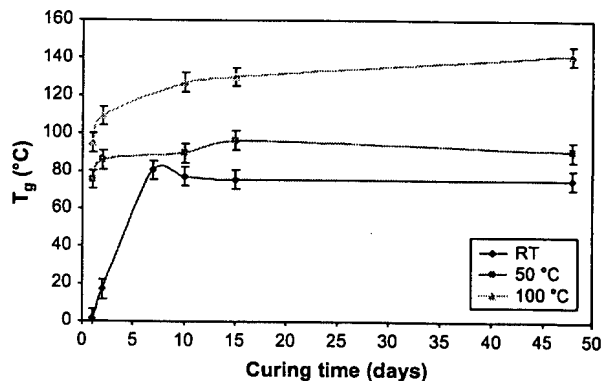
Fig. 5. MDSC of the 1 + 4 adhesive cured between Cu plates as a function of curing time (room temperature; glued samples were broken apart approximately 1 min before MDSC analysis to avoid changes in behaviour caused by water absorption).
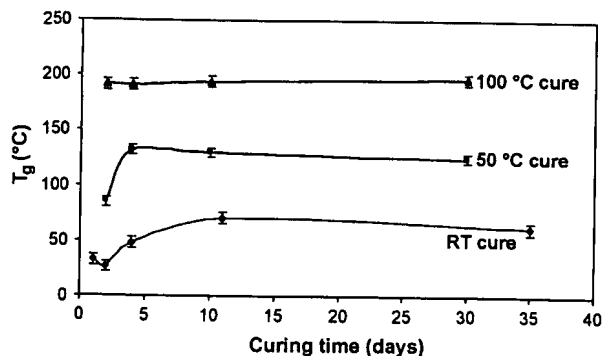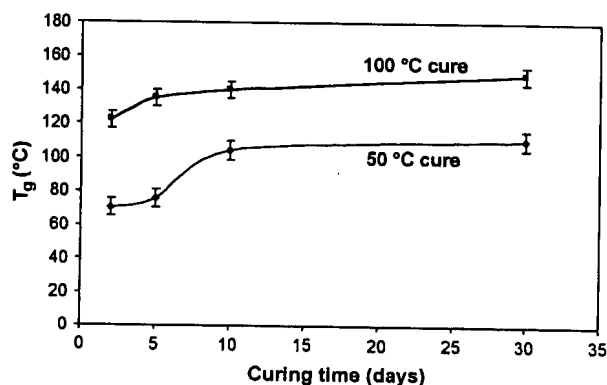
Fig. 6. Evolution of T_g with curing time of 1 + 4 at different curing temperatures determined by DMA single cantilever measurements.

incubated at 50 °C and 100 °C, but required 10 days for curing at room temperature. The drop in apparent T_g after 35 days was probably due to the plasticizing effect of adsorbed water.

Thus, polymerisation of 1 + 4 (solvent free between brass plates) produced materials with unusual T_g values significantly higher than the curing temperature. This is very unusual, as discussed below, for bulk (solvent free) polymerisation reactions.

3.2. Thermal properties of mixture 5 + 6

The thermal properties of polymers made from monomers 5 and 6 were investigated in the same manner, giving similar results. Thus, Fig. 7 shows the evolution of the T_g as a function of the curing time for materials made at 50 °C and 100 °C. At room temperature, curing occurred to only low T_g after 15 days, and so was not further analysed. As with 1 + 4, the final T_g for 5 + 6 is reached after 10 days of cure at elevated temperature. At 50 °C, this final T_g was found to be 60 °C higher than the curing temperature, and at 100 °C, T_g increased to 48 °C above curing temperature.

4. Discussion

The copper(I)-catalyzed AAC reaction between diazides and trialkynes is expected to produce highly crosslinked polymers which can be considered as thermosets. When

Fig. 7. Evolution of T_g with curing time of 5 + 6 at different curing temperatures as determined by DMA single cantilever measurements.

a thermoset undergoes polymerisation and crosslinking in the absence of a solvent its glass transition temperature increases with degree of polymerisation until either (i) it becomes fully cured with a glass transition temperature less than the curing temperature or (ii) the material vitrifies before fully cured and the rate of reaction becomes diffusion controlled and extremely slow. In the latter case the final glass transition temperature, at least for epoxies, is between 10 °C and 25 °C above the curing temperature [30,31]. In physically large items, apparently higher T_g's have often been observed but that is just because the reaction exotherm increases the temperature of the material being cured to significantly above the applied temperature.

Larger differences between the T_g and the curing temperature have been reported, in one case in an epoxy [32], however, it has been suggested that the high T_g in this case was the result of thermal degradation rather than curing [33]. A glass transition temperature 40 °C above the cure temperature was recently obtained by the use of reactive additives in a polyarylene, however, there was no discussion of the curing mechanism [34]. Glass transition temperatures 70 °C [33] to 100 °C [35] above the cure temperature have been reported in highly crosslinked, radically polymerised systems cured by photopolymerisation. It was suggested that the large differences between T_{cure} and T_g were obtained in these systems because (i) they cure by a chain growth polymerisations and (ii) very inhomogeneous networks were formed. More diffusion is expected in the vitreous state in chain growth polymerisations because a considerable amount of monomer can remain up to high conversions whereas that does not happen in chain growth polymerisations. In addition it was suggested that the active radicals may maintain a relatively high mobility in the glass through hydrogen transfer thus the reaction continues to occur deep into the glassy state. Network inhomogeneity is believed to increase T_g–T_{cure} because regions of high mobility will still exist in the glass permitting radicals, monomers and pendant double bonds to still diffuse and react.

The materials described here have a final T_g that is up to 50–60 °C higher than the curing temperature, well above the normal range of 10–25 °C seen in step growth polymerisation systems. Furthermore, this phenomenon appears to be general: the core structures of monomers 1 and 4 (aromatic rings) vs. 5 and 6 (pentaerythritol) (Scheme 1, Fig. 1) are quite different, and yet the T_g–T_{cure} relationship is similar. Other combinations of multivalent azides and alkynes linked by Cu(I) catalysis have also been found to behave similarly. Under normal circumstances, T_g of some thermosets can be up to 20 °C higher than the curing temperature due to bulk exothermic effects, the reaction exothermicity raising the temperature of the environment above that of the external medium as discussed above. The adhesive samples were extremely unlikely to experience bulk heating, since they were thin films (average 0.12 mm thickness) formed in slow polymerisations between metal sheets. Note, too, that such a high T_g polymer takes from 2 to 10 days to reach its final glass transition temperature when prepared on copper-containing surfaces with no added catalyst. At this stage the amount of solvent is very low.

This suggests that the curing reaction still proceeds even if the material is glassy and not much chain or monomer mobility is possible.

In the system described here, unlike the situation described in Ref. [35], the G' and tan δ peaks are not unusually wide, so the large difference between T_{cure} and T_g cannot be explained by inhomogeneity of the network. Instead, the mechanistic nature of the AAC process may be at least partially responsible for the observed high T_g values by creating chain crosslinks with unusual facility. Triazoles have good thermodynamic affinity for Cu(I) ions and yet the Cu–triazole interaction is kinetically labile. Cu ions should therefore be able to move readily from one triazole binding site in the developing network to another, even when the network is in the glassy state, in a similar way to radical diffusion in addition polymerisations. Cu–triazole complexes are also known to be good catalysts for the AAC reaction [36]. These factors combine to make it possible for Cu ions to migrate through the structure to create local "hot spots" of catalytic reactivity, especially in areas of the polymer matrix in which multiple triazoles have already been formed. The liberation of energy that attends every azide–alkyne cycloaddition event (exothermic by approximately 50 kcal/mol) may also induce increased local motion of polymer chains in the vicinity of the catalytic hot spots.

The nature of the triazole unit may also be important. We have found in previous work that linear polymers assembled from diazides and dialkynes, such as **3**, are unusually brittle and poorly soluble, although their T_g values are reasonably close to their curing temperatures [22]. The mechanical and solubility properties of these linear condensation polymers are likely due to the properties of the triazole unit in the main chain of such materials. 1,2,3-Triazoles have large dipole moments, good hydrogen bond accepting capability, and a pseudoaromatic nature that allows for π-stacking. Each of these factors can contribute to strong noncovalent interactions, and we suggest that associated domains are thereby formed very easily, leading to brittle materials in the case of the linear polymers and perhaps to associated domains of unusual stability during the formation of crosslinked networks from **1 + 4**. 1,2,4-Triazoles are quite common in the literature of metal coatings and adhesives [26,28,37], but no reports of unusually high T_g values have been made for these systems. This is significant because 1,2,4- and 1,2,3-triazoles share an affinity for binding metal surfaces through N–σ donor interactions, but 1,2,3-triazoles have much higher dipole moments than that of the 1,2,4-isomers.

5. Conclusion

High glass transition temperature thermosets have been formed between copper or brass plates by the copper(I)-catalyzed AAC reaction of diazides and a tri- and quad-alkyne. The material can show a T_g up to 50–60 °C higher than the curing temperature after curing for several days. This behaviour can be seen for different curing temperatures from RT to 100 °C. Solvent evaporation is not an issue as the solvent

evaporates very quickly leaving an insignificant amount in the adhesive joint. The materials are unusual as they continue to react, showing some local mobility, in spite of being in the glassy state. We can then assume that the curing rate of the sample is mainly dependant of the curing time and temperature. From a practical point of view, such materials may be useful as corrosion inhibitors or adhesives on copper or copper based substrates.

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existence of a hamiltonian path, but that will reveal nothing about it[4]. If Petra is mistaken or in a cheating mood, Virgil will spot a contradiction in her responses and reject her 'proof'. If Petra is correct, however, not only will Virgil believe her claim, but he will learn nothing else that he could not figure out on his own without Petra's help. Better still, no amount of deviousness in posing the questions on Virgil's part can alter that fact. Zero knowledge is a mechanism for enforcing honest behaviour on both sides: cheating would bring no benefits to Virgil and it would expose Petra to the embarrassment of getting caught. Little wonder that zero knowledge is often held up as the 'holy grail' of secure computing.

But do zero-knowledge proofs even exist? Under widely accepted assumptions, the answer is yes for any NP-complete problem[5]. Were Petra to hand over the hamiltonian path, Virgil would learn more than the mere truth of her claim: he would know the itinerary itself, something, we just argued, he could not find on his own. To achieve zero-knowledge status requires, as so often in life, dialogue, commitment, and a bit of luck. To keep Petra from deceiving him with inconsistent answers, Virgil will ask her to commit to her claim once and for all. This is the software equivalent of hiding the hamiltonian path in a locked box that neither party can access until both jointly decide to open it. In fact, the path must be relabelled randomly, so that merely looking at it will not help Virgil trace it in the original map. Why so

much suspicion? Because distrust is the very ailment that zero knowledge seeks to cure: if Virgil trusted Petra, after all, her good word alone would be sufficient and no proof would be needed.

The surprise is that, to enforce honesty among distrustful parties, randomness must be thrown into the mix. A nasty side effect is that the conversation might go awry and lead Virgil wrongly to conclude that a hamiltonian path exists when none is to be found. This should happen with a probability of below 50%. (Correct claims will never be rejected, however.) Isn't this being inordinately lax? No: by repeating the dialogue a few dozen times, one can easily reduce the error probability to one in a trillion.

For technical reasons, to carry on several such conversations at the same time, as might happen on the Internet, is a big no-no. The issue is subtle, but to see why concurrency facilitates deception is not. Ever care to 'beat' your local chess-club champ? Here is how you do it: arrange for Garry Kasparov to play a match with you while you play with the local champion simultaneously — and do it online so neither one can see what you're up to. The trick is to feed each player the other's moves: in all likelihood, you will lose to Kasparov, but win against your neighbourhood champion.

The beauty of Barak and Sahai's work[2] is that they are able to overcome the pitfalls of concurrency by deploying a sophisticated

arsenal of cryptographic techniques. One of them is to relax the definition of zero knowledge by enhancing Virgil's power to simulate any potential dialogue with Petra. Another is to squeeze long messages into shorter ones without losing their security properties. Although fairly complex, the Barak–Sahai technology takes us one step closer to true security on the Internet.

In 1962, US President John F. Kennedy dispatched Dean Acheson to Paris to offer Charles de Gaulle photographic evidence of Soviet missiles in Cuba. The French president declined to see it, saying: "The word of the president of the United States is good enough for me." Zero knowledge is so blissfully easy in a climate of trust. The challenge is to deal with liars and cheaters. The continuing work of those such as Barak and Sahai[2] is giving us new tools to do that on the Internet. ■

Bernard Chazelle is in the Department of Computer Science, Princeton University, 35 Olden Street, Princeton, New Jersey 08540-5233, USA.
e-mail: chazelle@cs.princeton.edu

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CHEMICAL BIOLOGY

Dressed-up proteins

Gijsbert Grotenbreg and Hidde Ploegh

Proteins aren't just defined by their constituent amino acids — structural modifications can yield complex mixtures of protein forms. An approach that controls the addition of such modifications may help to define their role.

Polypeptides freshly minted from the cell's protein-assembly apparatus are by no means ready for active service. Both the peptide backbone and its side chains may need to be altered by post-translational modifications (PTMs), the covalent attachment of chemical groups that change the properties, and hence the function, of newly generated proteins. PTMs also control the degradation of aberrant proteins and those at the end of their lifespan. Such modifications dramatically expand the compositional and functional complexity of these molecules. Not satisfied with leaving everything to nature's whim, chemists are taking great strides in developing PTM-mimics. On page 1105 of this issue[1], van Kasteren et al. describe strategies that offer precise control over the attachment of carbohydrates and other PTM-mimics to polypeptides.

In nature, any given protein may exist as a mixture of forms, each incorporating different PTMs. Assessment of the contribution made by each PTM to that protein's function demands that the individual components of these mixtures should be isolated and identified. But separating the constituents from these complex — and possibly dynamic — mixtures is a dauntingly arduous task. The ability to uniformly modify proteins at specific sites in the molecule, using chemical approaches that mimic the original PTM, is therefore essential for progress in this area.

A few examples of this approach are already known. One clever and synthetically straightforward example was reported recently[2], in which the side chains of cysteine amino acids in proteins were selectively targeted to mimic a particular PTM — the modification of lysine

amino-acid side chains with methyl groups. The resulting molecules were used to assess the role of this PTM in DNA–protein complexes found in cell nuclei.

One intricate form of PTM that remains a prize target for chemists is the attachment of carbohydrates — glycans — either to nitrogen atoms (N-linked) in the side chains of asparagine amino acids, or to oxygen atoms (O-linked) on the side chains of serine or threonine. In living cells, glycosidase and glycosyl transferase enzymes first trim N-linked glycans, then extend them with sugars that can branch in several directions, generating numerous variations on a theme. Glycosyl transferases also act on O-linked glycans, imposing similar extensions and modifications. To add to the complexity, each sugar can bear different chemical groups, and the linkages between sugars have specific orientations. Many biological processes, such as cellular differentiation and development, cell adhesion, immune surveillance and inflammation, rely to varying degrees on the correct decoration of proteins with such glycans.

Van Kasteren et al.[1] have now responded to the clarion call for improved synthetic methods to modify proteins. Not only have they installed carbohydrate PTM-mimics at specific locations in a protein, but they have also incorporated another important motif — a sulphated
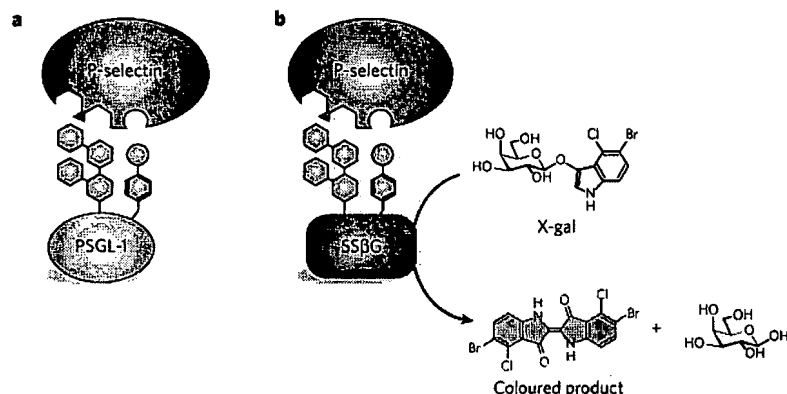
Figure 1 | Dressing up proteins. a, The protein P-selectin recognizes and binds to its ligand, PSGL-1, using covalently bound chemical groups (known as post-translational modifications, PTMs) at the ligand's surface. The PTMs are a carbohydrate (sialyl-Lewis-X, orange hexagons) and a sulphated amino acid (sulphotyrosine, blue hexagon with circle attached). **b**, Van Kasteren et al.[1] show that the unrelated SSβG protein can be fitted with mimics of these PTMs so that it is recognized by P-selectin. SSβG enzymatically converts X-gal molecules into a coloured product. This reporter function of the modified SSβG was exploited to visualize P-selectin in tissue samples.

carbohydrate known as sialyl-Lewis-X — to proteins is not unprecedented[7]. But what makes van Kasteren and colleagues' work unique is that two distinct PTMs have been installed on a single polypeptide. This successful application of two distinct chemical processes, involving reaction centres that recognize each other, but which ignore the dense concentration of chemical groups surrounding them, represents a formidable achievement.

As a target to which they could apply their technique, van Kasteren et al.[1] chose the protein P-selectin, whose presence in tissue is indicative of inflammation and is also a useful hallmark of cerebral malaria. P-selectin recognizes another protein, PSGL-1, by means of two distinct PTMs — a sulphated tyrosine residue and a sialyl-Lewis-X carbohydrate — found at separate locations on the PSGL-1 backbone (Fig. 1). The authors took an enzyme (Sulfolobus solfataricus β-glycosidase, SSβG) that normally doesn't bind to P-selectin, and decorated it with PTM-mimics to resemble PSGL-1. The presence of SSβG can be revealed when it converts a molecule known as X-gal to a blue product. Would SSβG, dressed up as PSGL-1, now allow the visualization of P-selectin? The answer was a resounding yes. The mere presence of the PTM-mimics positioned appropriately on the surface of SSβG allowed it to be recognized by P-selectin, as reported by the blue enzymatic reaction product. In a convincing demonstration of the technique's utility, PTM-bearing SSβG protein was used to visualize chronic inflammation and cerebral malaria pathology in tissue sections.

This work artfully brings together several chemical tools to obtain pure proteins

tyrosine amino acid — at another defined site in the same molecule. To accomplish such a feat of directed chemistry, a creative synthetic strategy was required. The authors specifically appended their sulphotyrosine mimic to the side chains of cysteines using a selective 'disulphide exchange' reaction. To attach the carbohydrate PTM-mimic, van Kasteren et al.[1] incorporated chemical groups (alkynes and azides) into the glycan and the protein. These groups react exceptionally well with each other under near-physiological conditions[3,4], but are indifferent to all other groups present.

To target their PTM-mimics to precise locations, the authors used genetically engineered

bacteria to create proteins either with cysteine or with azide-containing or alkyne-containing amino acids at the desired surface positions. Azide-containing and alkyne-containing amino acids do not occur in nature, so these were incorporated by tricking the cell's biosynthetic machinery into installing them at positions where a methionine amino acid would otherwise have occurred.

The uptake and incorporation of azide-containing building-blocks into biomolecules has previously proven its worth, as azide groups can be selectively labelled by chemical means[5,6]. Similarly, the tethering of carbohydrates — from simple glucose to the complex

APPLIED PHYSICS

Weight inside

How can tiny particles such as molecules be weighed? A cantilever, if small and flexible enough, will bend under the weight of a molecule adsorbed onto its surface. A rather more sensitive mass measurement is given by the shift in the cantilever's resonance frequency as mass is adsorbed. This tiny resonance signal can be read out electronically by integrating the cantilever onto a silicon chip.

Previous demonstrations of chip-based mass sensors include the detection of a single virus weighing as little as 9.5 femtograms (1 femtogram is 10^{-15} grams) in air (A. Gupta et al. Appl. Phys. Lett. **84**, 1976–1978; 2004), and weighing a cluster of about 30 xenon atoms — equivalent to a mere 7×10^{-21} grams — in a vacuum (Y. T. Yang

et al. Nano Lett. **6**, 583–586; 2006). But where the particles to be weighed are suspended in solution (as is the case, for example, in many biological settings), the technique hits a snag: the viscosity of the fluid damps the resonator, and significantly decreases its sensitivity.

Elsewhere in this issue, Scott Manalis and colleagues present what might be described as a radical solution to the problem: putting the fluid inside the resonator (T. P. Burg et al. Nature **446**, 1066–1069; 2007). They have designed a vacuum-sealed silicon microcantilever with hollow channels (pictured), connected to pressure-controlled inlets and outlets for fluid delivery.

The device works in two modes. In the first, which is particularly suited to selective detection of biomolecules,

a solution is continuously run through the channels and particles can adsorb on the channels' inner surface — which must be specially prepared for the purpose. The authors demonstrate how the mass change can be followed by monitoring shifts in the resonance frequency of the cantilever in real time as proteins in solution become bound to appropriate receptor molecules that have been grafted onto the tube surface.

In a second mode, particles are detected in transit through the channels; this is useful for weighing particles in dilute solutions. An experiment in this mode determined the distribution in masses, with a resolution of one femtogram, for two types of live bacterial cell, of average masses 110 and 150 femtograms.
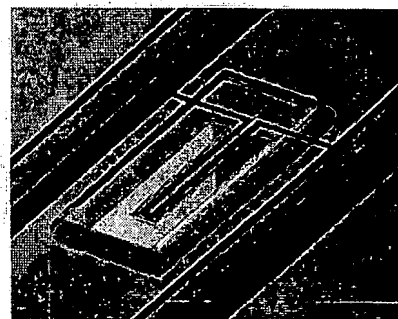
The work is an example of the steady progress that is being made in designing practical, inexpensive and portable lab-on-a-chip diagnostic devices. Although further advances are required to demonstrate a 'killer application' for Burg and colleagues' fluidic sensor — a medically relevant specific detection of viruses in blood samples, for example — it is already an elegant method for weighing tiny particles in solution.
Liesbeth Venema

carrying specific, complex-carbohydrate-based PTMs. It remains to be seen whether proteins other than conveniently sized reporters such as SSβG will tolerate such manipulation with similar ease. Nevertheless, by combining the genetic manipulation of proteins with chemical reactions to selectively install biologically active structures, van Kasteren *et al.*[1] have taken a crucial step towards unravelling the contributions of complex PTM patterns to protein function. ∎

Gijsbert Grotenbreg and Hidde Ploegh are at the Whitehead Institute for Biomedical Research and the Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, Massachusetts 02139, USA.
e-mail: ploegh@wi.mit.edu

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MATHEMATICAL PHYSICS

Added dimensions to grain growth

David Kinderlehrer

A long-standing mathematical model for the growth of grains in two dimensions has been generalized to three and higher dimensions. This will aid our practical understanding of certain crucial properties of materials.

Most engineered materials, including metals and ceramics, are polycrystalline — that is, they are composed of myriad, small, crystalline grains, separated by interfaces or boundaries (Fig. 1). Yet despite the ubiquity of such grains, we know surprisingly little about how they grow. On page 1053 of this issue, MacPherson and Srolovitz[1] supply a precise answer in three dimensions — indeed, in any number of dimensions. The result is not merely novel, but also intellectually exciting: and it is exciting both for materials scientists, who now know that this question has a succinct and elegant answer, and for mathematicians, who can witness recondite methods of integral geometry resolving a question of material significance.

The study of crystalline microstructure is ancient. Our ancestors fashioned stone implements from tool-stones that had special microstructures, and the excavation of such tool-stones has informed our ideas about human migration[2]. In the world of modern materials, cellular networks are implicated in many, varied material properties at many different length scales, including lifetime properties such as fracture toughness, and functional properties such as electrical conductivity. Preparing arrangements of grains and boundaries suitable for a given application is a central problem of materials science: the problem of microstructure.

Over time, and especially with exposure to elevated temperatures, grain boundary networks coarsen, and thus grains grow. Promoting or preventing this process is of paramount technological importance in many engineered materials. Simultaneously, a grain configuration must completely fill space and, in some way, minimize energy[3]. In other words, it is subject to both topological and thermodynamic constraints.

The simplest coarsening mechanism is local: each point on a boundary wall moves perpendicularly, with a rate proportional to its surface tension. But to understand the evolution of a network, this microscopic description is unsatisfactory: one cannot inspect the surface tension at every point. Hence the central question: how does the volume of a cell (which is easier to measure) change? In the two-dimensional case, the answer was given by John von Neumann[4] in 1952 and William Mullins[5] in 1956. Often just called the '$n - 6$ rule', it states that the rate of change of area, dA/dt, of a two-dimensional cell with n sides is given by $dA/dt = \gamma(n - 6)$, where γ is a material constant. Thus, cells with more than six sides grow (positive change in area with time) and those with fewer than six sides shrink (negative change). The familiar pattern of regular hexagonal tiles is an equilibrium configuration.

The equation is, in fact, a disguised version of the familiar geometric fact that the sum of the exterior angles of a polygon is 2π (360°). As an irregular cell grows, its sides are assumed to maintain equal surface tension and to meet at vertices of interior angle 120° — as indeed they do in the regular hexagon. It is interesting to note here that the dimensionality of the measurement implied in the equation (the number of sides, which has no dimension) is two less than what is being measured (a two-dimensional change in area). The ($n - 6$) notation contains all the ingredients for measuring and counting cell features that extend to the three-dimensional structures.

In this respect, MacPherson and Srolovitz's result in three dimensions has a familiar face, as it were. It takes the form

$$dV/dt = \gamma\left(\sum_{i=1}^{\bullet} e_i - 6L\right)$$

and thus relates the rate of change of (three-dimensional) volume, dV/dt, of a cell to two
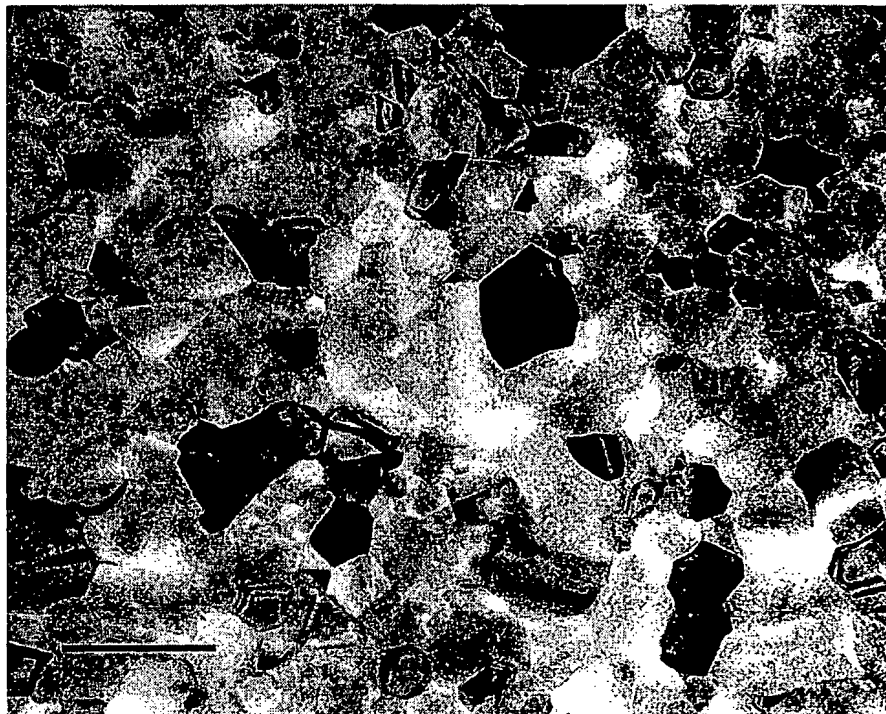
Figure 1 | With the grain. The way in which the area of a two-dimensional granular structure — here, a transmission electron micrograph of an aluminium thin-film structure — varies with time has been known for more than 50 years. Only now have MacPherson and Srolovitz[1] managed the jump to three and higher dimensions. (Scale bar, 200 nm; image courtesy of K. Barmak.)

A chemoselective approach for the accelerated synthesis of well-defined dendritic architectures†

Per Antoni,[a] Daniel Nyström,[a] Craig J. Hawker,[b] Anders Hult[a] and Michael Malkoch*[a]

A chemoselective and layered growth approach has been developed for the synthesis of dendrimers, combining Click chemistry with traditional esterification/etherification reactions, without the need for activation steps and with excellent overall yields.

Dendrimers are highly branched polymers that have gained increasing attention in a variety of nanotechnology applications from microelectronics to biomedical devices.[1] It is generally known that dendrimers are time consuming and expensive to construct using traditional multi-step techniques which limits their commercial availability to PAMAM®, DAB®, Phosphorous PMMH and 2,2-bis(methylol)propionic acid (bis-MPA) dendrimers.[2,3] Nevertheless, their well-defined, modular structure with high functional group density and 3-dimensional shape make these synthetically challenging scaffolds extremely attractive molecular targets. For example, PAMAM dendrimers have been modified with sulfonic acid end groups to afford a novel dendritic HIV/ AIDS[4] drug while the same PAMAM scaffolds with hydrophilic oligo(ethylene glycol) end groups have been used as pore generating agents for the development of dielectric layers for advanced microelectronic devices.[5]

[a] Fiber and Polymer Technology, Royal Institute of Technology, Teknikringen 56-58, SE-10044 Stockholm, Sweden.
E-mail: Malkoch@polymer.kth.se; Fax: +4687908283;
Tel: +4687908768
[b] Materials Research Laboratory (MRL), University of California Santa Barbara, California 93106, USA. E-mail: Hawker@mrl.ucsb.edu; Fax: +18058938797; Tel: +18058937161
† Electronic supplementary information (ESI) available: Experimental procedures and characterization. See DOI: 10.1039/b703547k

Traditionally, two synthetic strategies are available for the preparation of dendrimers, either the divergent[6] or convergent[7] growth approach. Both strategies yield high generation dendritic structures from AB_x-monomers through repetitive growth and activation steps. To achieve 'perfect' structures, the chosen chemistry needs to be essentially quantitative as inefficient reactions lead to defects in the dendritic structures and may give rise to tedious purification procedures. As a result, new synthetic strategies and versatile coupling reactions that will enable the preparation of dendritic macromolecules under accelerated conditions and with unprecedented control are of significant interest. A reaction that has gained tremendous attention since it was reported by Fokin and Sharpless in 2002 and which fulfills all the criteria for the construction of dendritic structure is the Cu catalyzed Click reaction of azides with terminal alkynes.[8] This reaction is highly efficient and occurs under benign conditions in the presence of other reactive groups with no byproducts. Due to its orthogonal, robust nature, this example of Click chemistry has attracted significant attention in the materials science community, especially for the construction of complex polymeric materials.[9] For example, the construction of multifunctional AB-diblock dendrimers with both targeting ligands and imaging agents for investigating the binding with cell surfaces was successfully accomplished in high yields using Click chemistry.[10] Nonetheless, while the introduction of new chemical reactions has greatly enabled the efficient construction of dendrimers,[11] the synthetic approaches employed still involve the traditional multi-step, divergent or convergent approaches.

To greatly increase the availability of functionalized dendrimers and to accelerate their use in a variety of applications the development of new methodologies for their synthesis is required. The requirements for such an approach are that it should involve

Fig. 1 Graphical representation of the number of synthetic steps required for the preparation of a Gen = 4 dendrimer using an accelerated chemoselective synthesis (top) versus a traditional divergent dendrimer synthesis (bottom).

fewer reaction steps, be quantitative in nature, compatible with a variety of functional groups and occur under mild reaction conditions. This can be accomplished through the synthesis of AB_2- and CD_2-monomers that selectively react with each other and employ reactions that proceed in very high yield (Fig. 1). If this chemoselectivity can be achieved, AB_2 + CD_2 systems lead to generation growth involving only a single reaction with little or no purification required. The orthogonality required in the selection of the A, B, C and D functional groups also dictates a high degree of flexibility in the incorporation of reactive functional groups.

This communication describes the development of an accelerated growth approach[12] to dendrimers based on the combination of Click chemistry with traditional esterification/etherification reactions, for the synthesis of dendrimer derivatives of the classical Fréchet-type and bis-MPA dendritic macromolecules. These structures were chosen due to their use in dendrimers as well as to demonstrate the modular nature of this synthetic approach and its applicability to different chemistries and monomer units. To illustrate the efficiency of the accelerated approach methodology the synthesis of analogs of Fréchet-type dendrimers was initially examined through the combination of Click and etherification reactions. The required monomer units for one-step generation growth are 1-(bromomethyl)-3,5-bis(prop-2-ynyloxy)benzene, 3, as nominally the AB_2-monomer, and 5-(azidomethyl)benzene-1,3-diol 5, as the CD_2-monomer unit.

In this case, orthogonal reactivity is achieved by the Click reaction of the terminal acetylenes with the azide group of 5 in the presence of the phenolic moieties of 5 followed by the etherification of the terminal phenolic groups of the growing dendrimer, 7, with the bromomethyl group of 3 with the terminal acetylenes of 3 giving rise to the third generation dendrimer 8, containing

24 terminal acetylene groups (Scheme 1). This synthetic strategy allows the divergent preparation of a fourth generation dendrimer 9, containing 48 terminal phenolic groups, in only four steps and in multi-gram quantities with an overall yield of 70% from the starting triphenol, resulting in a layered dendritic block copolymer with alternating layers of benzyl ether, triazole and benzyl ether groups. The orthogonality and efficiency of this strategy were demonstrated by monitoring each reaction with NMR and Maldi-TOF techniques. As can be seen in Fig. 2, the growth progress of the fourth generation dendrimer 9 was monitored by ^{1}H NMR and the Click reaction was found to reach completion within 12 h at RT. This was confirmed since the peak corresponding to the acetylene terminal groups at 3.55 ppm in DMSO was not detectable. It should be noted that a traditional divergent growth approach would require at least eight steps with multiple purification procedures for the synthesis of a similar fourth generation structure.

Moreover, the dendritic products obtained have alternating end groups, either acetylene or phenolic, depending on the generation number which allows for convenient and efficient post modifications without the need for elaborate activation of dormant species. To further demonstrate the versatility of this concept, the accelerated synthesis of analogs of bis-MPA type dendrimers was addressed. Traditionally, bis-MPA dendrimers require growth/activation steps involving esterification and deprotection reactions. To develop bis-MPA type dendrimers via this accelerated approach, esterification reactions were coupled with Click chemistry. Initially, a bis-MPA based AB_2-monomer containing both an acyl chloride and azide functionalities was designed, 13, and coupled with the corresponding CD_2-monomer, 15, which is the propargyl ester of bis-MPA (Fig. 3). Generation growth was
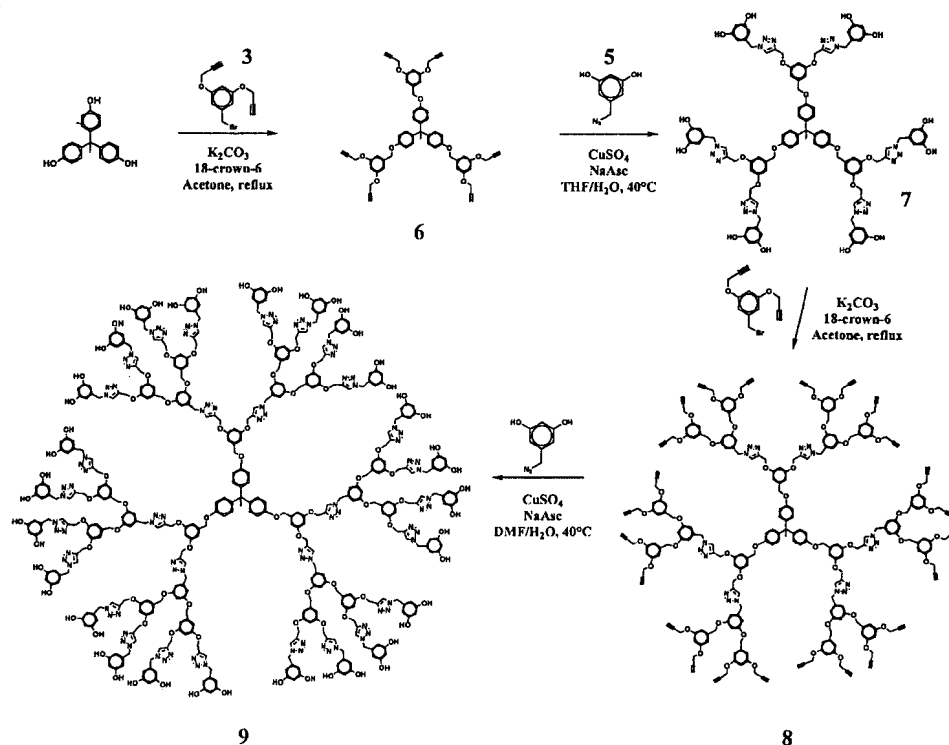
Scheme 1 Chemoselective synthesis of a Gen = 4 Fréchet-type dendrimer using an accelerated AB_2 + CD_2 approach.
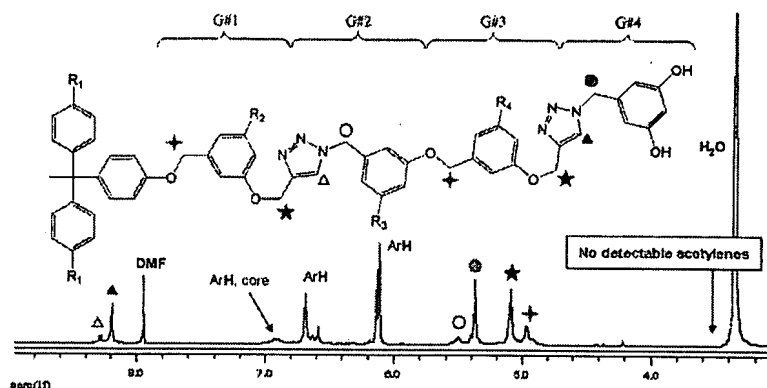
Fig. 2 Crude ^{1}H NMR spectra of a Gen = 4 Fréchet-type dendrimer, **9**, containing 48 terminal hydroxyl groups.

accomplished *via* an esterification reaction of **13** with the trisphenolic hydroxyl core followed by a Click reaction of **15** to yield a second generation bis-MPA type dendrimer with 12 terminal hydroxyl groups. Repetition of this sequence of reactions gives the fourth generation dendrimer, **19**, which again is obtained in excellent yield with high levels of purity after only four reaction steps.

The distinct difference between this chemoselective accelerated approach and the traditional strategies for dendrimer synthesis is

the repetitive utilization of two highly versatile reactions in sequence which avoids numerous activation or deprotection steps. As a result, growth is not only accelerated but a much richer family of functionalized dendritic structures can be prepared. This versatility is demonstrated by the accelerated synthesis of two different types of dendrimers, analogs of bis-MPA-type and Fréchet-type dendrimers. The simple and orthogonal nature of these strategies also allows the synthesis of a wide variety of other macromolecular architectures and hybrid materials where monomers from different families are combined to generate new materials with unique features.

Fig. 3 Accelerated synthesis of a Gen = 4 bis-MPA dendrimer synthesized in only four steps from **13** and **15**.

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Bringing Efficiency to Materials Synthesis:
The Philosophy of Click Chemistry

Craig J. Hawker,[A,C] *Valery V. Fokin,*[B] *M. G. Finn,*[B] *and K. Barry Sharpless*[B]

[A]Materials Research Laboratory, University of California, Santa Barbara, Santa Barbara, CA 93106, USA.
[B]Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research
  Institute, BCC-315 10550 N Torrey Pines Road, La Jolla, CA 92037, USA.
[C]Corresponding author. Email: hawker@mrl.ucsb.edu

We open this Essay by paying homage to Sir John Cornforth, who was inspirational to the establishment of click chemistry as one of the most rapidly growing areas of research in materials synthesis. In two distinct ways, the influence of this giant of 20th century chemistry was of fundamental and practical importance. First, the philosophy of Professor Cornforth permeates modern materials chemistry. Cornforth saw himself as a molder of basic building blocks, '*As a carpenter or carver learns to work with the grain of wood or bone, I learned that each substance has its own nature and can be easy or difficult to handle according to the procedure chosen*'.[1] In addition, Cornforth appreciated the goal of efficiency in chemical synthesis, '*The ideal chemical process is that which a one-armed operator can perform by pouring the reactants into a bath tub and collecting pure product from the drain hole*'. Modularity and efficiency are inherent in polymer synthesis, but the explicit appreciation by Cornforth of the importance of both in the preparation of complex, multifunctional systems helped to inaugurate an important era of modern materials chemistry. Second, in 2002 the inaugural Cornforth Symposium was held at the University of Sydney and two of us (Sharpless and Hawker) were honored to be the inaugural lecturers. While polymer chemistry was recognized from the first as an important inspiration for (and potential beneficiary of) the click concept,[2] it was during the symposium and surrounding discussions that the real promise of click chemistry in materials synthesis first began to be appreciated. Initial scribbling of possible research directions quickly became firm outlines of a series of collaborative studies between the Scripps groups and the Hawker group, and these studies helped to lay the foundation for the exploitation of azide/alkyne coupling as a valuable new synthetic technique in synthetic chemistry. A two-way exchange of students between Scripps and UCSB/IBM allowed these studies to become reality with the fruit of this collaboration being the initial report of applying click chemistry to highly efficient dendritic materials synthesis requiring minimal purification.[3] Other papers further demonstrating the particular allure of click chemistry for materials synthesis quickly followed including the development of novel adhesives for Cu surfaces, a significant materials issue.[4]

When viewed as a whole, these initial studies demonstrated that the difficulties and challenges traditionally associated with the synthesis of well defined materials—controlling multiple reaction sites, quantitative conversion, purification issues, etc.—represent a significant opportunity for efficient and orthogonal strategies such as click chemistry.[5] The complete chemo- and regio-specificity, quantitative yields, and almost perfect fidelity in the presence of a wide variety of functional groups and reaction conditions that are the hallmarks of click reactions greatly enable the fabrication of well defined, multifunctional materials.[6] Given the influence of 'Kappa' Cornforth and the storied history of polymer science in Australia, it is, therefore, entirely appropriate that a Research Front on the application of click chemistry to the synthesis of functionalized polymers should be published in the *Australian Journal of Chemistry*.

The application of the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction—so far, the best example of click chemistry—to materials and polymer synthesis has sparked a true re-invigoration of interest in this widely studied and critically important field. Since the discovery of the CuAAC process in 2002,[7] more than 300 papers have described its use in materials and surface science. One might be tempted to ascribe this remarkable flurry of activity to the application of

a new reaction to accomplish things that can already be done. However, an examination of the literature shows that something more important is going on. Click chemistry not only allows particular new materials to be prepared, it opens up whole new avenues for materials in general to be prepared more efficiently. A perfect example is the area of biomaterials where the use of azide–alkyne coupling removes constraining considerations of protection/deprotection and cross-reactivity from the introduction of peptide sequences, sugar residues, etc.[8] In addition, click chemistry allows existing materials classes to be prepared with unprecedented efficiency and versatility opening up an array of new structures. A classic example is the area of block copolymers, where researchers have for decades tried valiantly, but ultimately unsuccessfully, to develop generally viable synthetic schemes for the coupling of chain end functionalized polymers to form high molecular weight, well defined products. The dream of taking bottles of stable, functionalized homopolymers from the shelf, and mixing them together under mild reaction conditions in eqimolar ratios to obtain high yields of block copolymers is now a reality thanks to click chemistry.[9] Thus click chemistry provides a valuable alternative to living polymerization methods such as anionic, ring opening, living radical systems where blocks are made through sequential polymerization of monomers rather than through coupling of preformed blocks.

The papers in this Research Front address many of the key features of click chemistry and present some of the myriad synthetic possibilities. Concrete examples are provided in an insightful review by Evans[10] which provides further evidence for the rapid adoption of these principles by the materials synthesis community. As elucidated by Evans, a significant opportunity in biomedical research is the conjugation of synthetic polymers with bioactive moieties.[11] Lutz demonstrates that the coupling of the 14-amino-acid TAT sequence for cellular transduction can be successfully accomplished at room temperature using click chemistry even though the two starting materials have markedly different properties.[12] In a similar fashion, the CAMD group exploited both RAFT polymerization[13,14] and click functionalization to prepare novel glycopolymers which are attractive materials for biomedical applications due to their hydrophilicity and biocompatibility.[15] By taking advantage of the orthogonality of click chemistry, Sumerlin[16] was also able to prepare trithiocarbonate-based vinyl RAFT agents which can be used to construct in a modular fashion, hyperbranched PNIPAM systems that show a lower critical solution temperature of 25°C. These stimuli-responsive materials also contain triazole units suitable for further non-covalent binding of therapeutic agents for controlled release applications. Finally in a tour de force, Matyjaszewski exploits the quantitative nature of the alkyne/azide coupling reaction to assemble multisegmented block copolymers that could not be previously prepared without Herculean efforts and rigorously characterizes these materials with triple detection, size exclusion chromatography.[17] In all of these cases, as well as many others, the use of a click reaction has allowed investigators to easily bring new functions and functional groups to well established platforms and methods—one usually does not need to reengineer one's favorite system to take advantage of click ligation methods.

In the near term, the success and impact of click chemistry on materials science will depend on understanding the full capabilities of the azide–alkyne reaction. Very few studies have so far tried to frame its limitations in terms of yield versus molecular weight for the starting polymers, efficiency in introducing and manipulating click functional groups, effect of other reactive

functional groups, etc. These features need to be defined for the true potential of click chemistry to be realized. In addition, the unique properties of the triazole ring, which are quite distinct from those of its precursor alkyne and azide groups, provide a rich vein of research that is currently under-appreciated. Thus far, most have regarded the triazole as an innocuous connecting unit, which it certainly is in many circumstances. However, the extraordinarily stable and aromatic nature of the triazole ring, along with its large dipole moment and demonstrated H-bonding capability, make it a functional group of great potential utility.

What are the challenges for the future? The click chemistry 'philosophy' embodies an attitude that is already second nature to polymer and materials chemists: *function* matters most, and tools that allow you to achieve function are to be prized. As such, click chemistry is not one specific reaction: any click reaction can have a similar or even greater impact as the CuAAC process has had. It is therefore well worth the effort of synthetic chemists to develop new transformations which satisfy all of the criteria of a click reaction. An expanded palate of such tools would be of major benefit to the materials and chemical communities alike. The flagship azide–alkyne process can also be improved. For example, the development of a more biocompatible catalyst would help materials scientists operate in the biological realm. In a different area, the development of a photochemically activated alkyne/azide procedure would enable a wide range of potential applications from thin films adhesives to cellular scaffolds to be addressed.

As always, Nature serves as both inspiration and challenge: the cell is a soup of giant molecules, interacting with breathtaking specificity and orthogonality to achieve complex and interacting functions. If we hope to engineer systems that even remotely approach the utility of Nature's components, our synthetic methods must improve, and generally without access to Nature's evolved synthetic machinery. In this context, getting 'simpler' means getting better: more and varied click reactions would allow synthetic chemists to move closer to the grand challenge of mimicking the functional sophistication of living processes.

Whatever the success in developing and fully exploiting a palate of click reactions, there is no doubt at all that the importance of reaction efficiency and facile chemistry is now a major theme and underlying consideration when the design and synthesis of organic materials is first contemplated. We expect that the efficiency and user friendliness of click reactions will continue to allow new functional materials with important commercial applications to be prepared. Just as importantly, perhaps, we hope that click chemistry removes synthetic barriers to the implementation of new ideas. It may sound trite, but one of the chief benefits of the click approach, at least for us, is to make materials synthesis *fun* by making it more accessible. We wish the readers of this Research Front the same joy.

Acknowledgment

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Catalytic Azide–Alkyne Cycloaddition: Reactivity and Applications
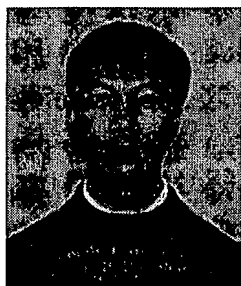
Dr. Peng Wu   Professor Valery V. Fokin

Peng Wu and Valery V. Fokin*
Department of Chemistry
The Scripps Research Institute
10550 North Torrey Pines Road
La Jolla, CA 92037, USA
Email: fokin@scripps.edu

Outline

1. Introduction

Copper(I) acetylides are postulated intermediates in a number of widely used organic transformations. The oxidative homocoupling of terminal acetylenes, discovered by Glaser in 1869,[1,2] is probably the most studied of these transformations. The scope of the reaction was extended in subsequent studies by Baeyer,[3] Straus,[4,5] Reppe,[6] and Eglinton.[7] Heterocoupling reactions of copper(I) acetylides, which do not involve oxygen, have been reported by Castro and Stephens (with aryl halides),[8,9] and Chodkiewicz and Cadiot (with bromoalkynes).[10,11] These copper-mediated cross-coupling reactions of alkynes have been extensively reviewed.[12-14]

1,3-Dipolar cycloaddition reactions have also been the subject of intensive research, most notably by Rolf Huisgen, whose work led to the formulation of the general concepts of 1,3-dipolar cycloadditions.[15] Dipolar cycloaddition chemistry has found widespread applications in organic synthesis and has been the subject of several reviews.

Given the extensive body of literature on the aforementioned topics, it is perhaps surprising that, with the exception of the disclosure of a copper-mediated synthesis of β-lactams from nitrones and alkynes by Kinugasa and Hashimoto in 1972,[16] the paths of copper acetylides and 1,3-dipoles did not cross, and the exceptional reactivity of copper acetylides towards organic azides was not discovered until the 21st century.[17,18] Even though organic azides have been used in synthesis for well over a century, their utility has been limited mostly to a facile introduction of the amino group into organic molecules. Other facets of their uniquely narrow reactivity profile remained largely unexplored until recently. In pioneering work on the development of small-molecule chemical reporters, Bertozzi recognized the nearly bioorthogonal properties of organic azides and the ease of their introduction into biological molecules. She elegantly exploited their reaction with phosphines (the modified Staudinger ligation) in metabolic oligosaccharide engineering studies.[19,20] Around the same time, the concept of "click chemistry" was introduced by Kolb, Finn, and Sharpless.[21] It was defined as an efficient approach to the synthesis of diverse compounds based on a handful of "near-perfect" (very selective, modular, high-yielding, wide in scope) carbon–heteroatom bond-forming reactions. The potential of organic azides as highly energetic, yet very selective, functional groups in organic synthesis was highlighted, and their dipolar cycloaddition with alkynes was placed among the top reactions fulfilling the click criteria. Nevertheless, it was only after the independent discovery of copper catalysis of this reaction by our group and Meldal's in 2002,[17,18] that its applications began to be reported, making it the most useful click reaction discovered so far. However, one should remember that the concept of click chemistry is clearly not limited to a single transformation.

In the few years since its discovery, the Cu-catalyzed azide–alkyne 1,3-dipolar cycloaddition (CuAAC) has been established as one of the most reliable means for the covalent assembly of complex molecules. It has enabled a number of applications in synthesis, medicinal chemistry, molecular biology, and materials science.

This review is not intended to be a comprehensive survey of the subject; it will focus instead on the fundamental aspects of the transformation, highlighting the advantages, potential, and limitations of CuAAC by using several representative examples. Additional reviews on this subject have recently been published.[22,23,24]

2. Copper-Catalyzed Azide–Alkyne Cycloaddition (CuAAC)

The fundamental thermal reaction, involving terminal or internal alkynes (Scheme 1, top), has been known for over a century (to the best of our knowledge, the first 1,2,3-triazole was synthesized by A. Michael from phenyl azide and diethyl acetylenedicarboxylate in 1893),[25] and has been most thoroughly investigated by Rolf Huisgen and co-workers in the 1950s to the 1970s.[26,27] The process is strongly thermodynamically favored ($\Delta H° = -45$ to -55 kcal/mol) due to the high potential-energy content of the two reaction components, but has a relatively high kinetic-energy barrier (ca. 26 kcal/mol for methyl azide and propyne[28]) that renders the reaction very slow at room temperature for unactivated reactants. Copper(I) catalysis[17,18] dramatically accelerates the reaction of azides with terminal alkynes (Scheme 1, bottom) and exhibits several features that make the catalyzed reaction unique among other block-ligation reactions.

1. The reaction is not significantly affected by the steric and electronic properties of the groups attached to the azide and alkyne reactive centers. For example, azides carrying a primary, secondary, or tertiary group; electron-deficient or electron-rich group; and aliphatic, aromatic, or heteroaromatic substituent usually react well with variously substituted terminal alkynes.

2. The reaction is unaffected by water and by most organic and inorganic functional groups; thus, all but eliminating the need for protecting-group chemistry.
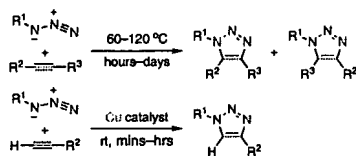
Scheme 1. The Uncatalyzed Thermal Cycloaddition of Azides to Alkynes Usually Requires Prolonged Heating and Results in Mixtures of the 1,4- and 1,5-Disubstituted Regioisomers. In Contrast, CuAAC Produces Only 1,4-Disubstituted-1,2,3-Triazoles at Room Temperature in Excellent Yields.
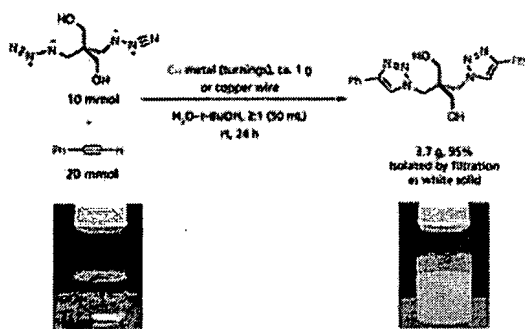
Ref. 17,28

eq 1

3. The rate of the Cu-catalyzed process is approximately 10^7 times that of the uncatalyzed version,[28] making the reaction conveniently fast in the temperature range of 0 to 25 °C. Furthermore, ligand-accelerated-catalysis effects[29] are also significant, resulting in further increases in the reaction rate.

4. The 1,2,3-triazole unit that results from the reaction has several advantageous properties: (i) a high chemical stability (in general, being inert to severe hydrolytic, oxidizing, and reducing conditions, even at high temperature), (ii) a strong dipole moment (5.2–5.6 D), (iii) an aromatic character, and (iv) a good hydrogen-bond-accepting ability.[30,31] Thus, it can interact productively in several ways with biological molecules, and serve as a replacement for the amide linkage in some circumstances.

2.1. Catalysts and Ligands

The robustness of CuAAC and its compatibility with most functional groups, solvents, and additives—regardless of the source of the catalyst—are evidenced by the number of experimental conditions that have been successfully employed for the reaction since its inception. The most commonly used experimental protocols and their advantages and liabilities are discussed below.

2.1.1. The Copper Catalyst

A number of different copper(I) sources can be utilized in the reaction. Copper(I) salts (CuI, CuBr) and coordination complexes (such as $[Cu(CH_3CN)_4]PF_6$,[17] $(EtO)_3P\cdot CuI$,[32] $[Cu(PPh_3)_3]Br$[33,34]) can be used directly. The latter have been particularly effective in organic solvents, in which cuprous salts have limited solubility.[35] However, Cu(I) is thermodynamically unstable and can be relatively easily oxidized to catalytically inactive Cu(II). The standard potential of the Cu^{2+}/Cu^+ couple is 159 mV, but can vary widely with the solvent and the ligand environment of the metal. Cu(II), itself, is an oxidant and can mediate the oxidative alkyne coupling reactions mentioned above, thereby impairing the efficiency of the cycloaddition and resulting in the formation of undesired byproducts. Therefore, when a copper(I) catalyst is used directly, whether by itself or in conjunction with amine ligands, exclusion of oxygen may be required to prevent these complications. As an alternative to oxygen-free conditions, a sacrificial reducing agent, for example ascorbate, can be used. Its combination with a copper(II) salt, such as the readily available and stable copper(II) sulfate pentahydrate or copper(II) acetate, is an economical and practical solution, and is the method of choice for preparative syntheses of 1,2,3-triazoles. Thus, "the aqueous ascorbate" procedure often furnishes triazole products in nearly quantitative yield and over 90% purity.[17]

Catalytic amounts of Cu(I) can also be introduced in the reaction through comproportionation of Cu(II) and Cu(0), thus further simplifying the experimental procedure. A small piece of copper metal (wire or turning) is all that is added to the reaction mixture, followed by shaking or stirring for 12–48 hours (eq 1).[17,36] Aqueous alcohols (MeOH, EtOH, t-BuOH), THF, and DMSO can be used as solvent. Cu(II) sulfate may be added to accelerate the reaction; however, this is not necessary in most cases, as copper oxides and carbonates (the patina), which are normally present on the surface of the copper metal, are sufficient to initiate the catalytic cycle. Copper nanoclusters, which are easily obtained and are air-stable,[37] and copper/cuprous oxide nanoparticles[38] have also shown excellent catalytic activity.

Although this procedure requires longer reaction times than the aqueous "ascorbate" variant, it usually yields very pure triazole products with low levels of copper contamination. Experimentally very simple, this protocol is particularly convenient for the high-

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throughput synthesis of screening libraries. The reaction is very selective, and triazole products are generally isolated in >85–90% yields, and can often be directly submitted for screening (**Figure 1**). When required, trace quantities of copper remaining in the reaction mixture can be removed with an ion-exchange resin or using solid-phase extraction techniques.

2.1.2. Auxiliary Ligands

Chemical transformations used in the synthesis of bioconjugates must be exquisitely chemoselective, biocompatible, and efficient. Despite the experimental simplicity and efficiency of the "ascorbate" procedure, it does not always perform well in its original form in bioconjugation applications. The reaction is not fast enough at very low concentrations of the reagents, and copper- or ascorbate-mediated degradation of the biological scaffolds has been observed. The ligand tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, 1; **Figure 2**), discovered soon after the disclosure of CuAAC,[39] significantly accelerates the CuAAC reaction and stabilizes the +1 oxidation state of the copper catalyst. In addition, it appears to sequester copper ions, thereby preventing damage to the biological molecules. After its utility was demonstrated by efficient attachment of 60 alkyne-containing fluorescent dye molecules to the azide-labeled cowpea mosaic virus,[40] it has been used numerous times in bioconjugation studies with nucleic acids, proteins, and cells. The sulfonated bathophenanthroline, 2, has been identified as a water-soluble and commercially available TBTA alternative.[41] In a recent study by Finn and co-workers, 2 was shown to be superior to TBTA for the efficient covalent attachment of small molecules to cowpea mosaic virus scaffolds,[42] eliminating the requirement for the large excess of a coupling partner (4–6 equivalents was usually sufficient) to achieve reasonable reaction rates and high yields. The drawback of this ligand is the increased sensitivity of the copper catalyst to oxidation, thus requiring the rigorous exclusion of oxygen from the reaction mixture.

2.2. Studies of the Mechanism of CuAAC

The intermediacy of copper(I) acetylides in CuAAC was postulated early on based on the lack of reactivity of internal alkynes. Soon thereafter, a computational study of the elementary steps of the sequence was performed. The initial computations focused on the possible reaction pathways between copper(I) acetylides and organic azides (propyne and methyl azide were chosen for simplicity).[28] The key bond-making steps are shown in **Scheme 2**. The formation of copper acetylide 4 (Step A) was calculated to be exothermic by 11.7 kcal/mol. This is consistent with the well-known facility of this step, which probably occurs through the intermediacy of a π-alkyne–copper complex. The π coordination of an alkyne to copper is calculated to move the pK_a of the alkyne terminal proton down by ca. 10 units, bringing it into the proper range to be deprotonated in an aqueous medium. A concerted 1,3-dipolar cycloaddition of the azide to the copper acetylide has a high calculated potential energy barrier (23.7 kcal/mol), thus the metal must play an additional role. In the proposed sequence, the azide is activated by coordination to copper (Step B), forming the intermediate 5. This ligand exchange step is nearly thermoneutral computationally (2.0 kcal/mol uphill when L is a water molecule). The key bond-forming event takes place in the next step (Step C), when 5 is converted to the unusual 6-membered copper metallacycle 6. This step is endothermic by 12.6 kcal/mol with a calculated barrier of 18.7 kcal/mol, which is considerably lower than the barrier for the uncatalyzed reaction (approximately 26.0 kcal/mol), thus accounting for the enormous rate acceleration accomplished by Cu(I). The CuAAC reaction is therefore not a true concerted cycloaddition, and its regiospecificity

is explained by the binding of both azide and alkyne to copper prior to the formation of the C–C bond. The energy barrier for the ring contraction of 6, which leads to the triazolyl–copper derivative 7, is quite low (3.2 kcal/mol). Proteolysis of 7 releases the triazole product, thereby completing the catalytic cycle.

The density functional theory (DFT) investigation described above was soon followed by an examination of the kinetics of the copper-mediated reaction between benzyl azide and phenylacetylene. This study revealed that, with catalytic Cu(I) concentrations under saturation conditions (rate independent of the alkyne concentration), the reaction was *second-order* in copper.[43]

$$\text{rate} = k[\text{alkyne}]^0[\text{azide}]^{0.2\pm0.1}[\text{Cu}]^{2.0\pm0.1}$$

The second-order dependence on Cu(I) is not unreasonable since most copper(I) acetylides are highly aggregated species,[44] and the second copper atom might be present, and in fact required, in the matallacycle-forming step. A recent DFT study suggests that this is indeed the case.[45]

2.3. The Cyclodimerization Phenomenon

The bimetallic requirement of CuAAC has manifested itself most dramatically in the ring closure of peptides prepared on the solid phase with azide and alkyne groups at opposite ends, as reported by Finn and co-workers (**Scheme 3**).[46] The 11-mer and 19-mer peptides,
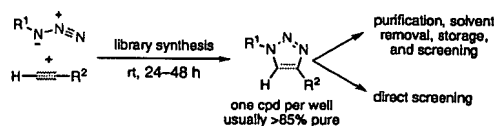
Figure 1. Protocol for the Synthesis and Direct Screening of Compound Libraries Using CuAAC.

Ref. 39–42
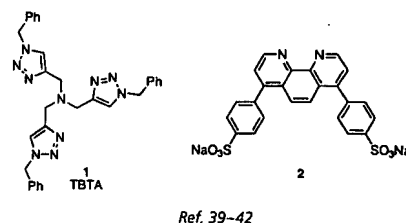
Figure 2. Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) and Sulfonated Bathophenanthroline Ligand for CuAAC.

Ref. 17,28
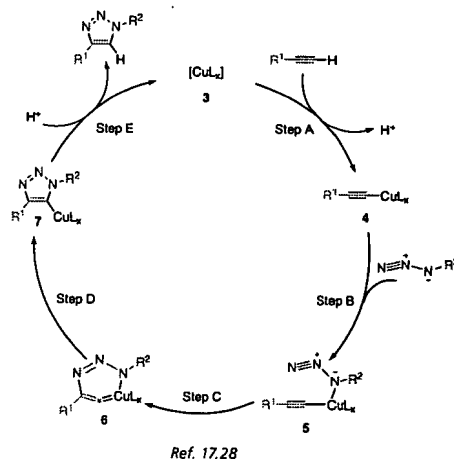
Scheme 2. Proposed Catalytic Cycle for CuAAC.
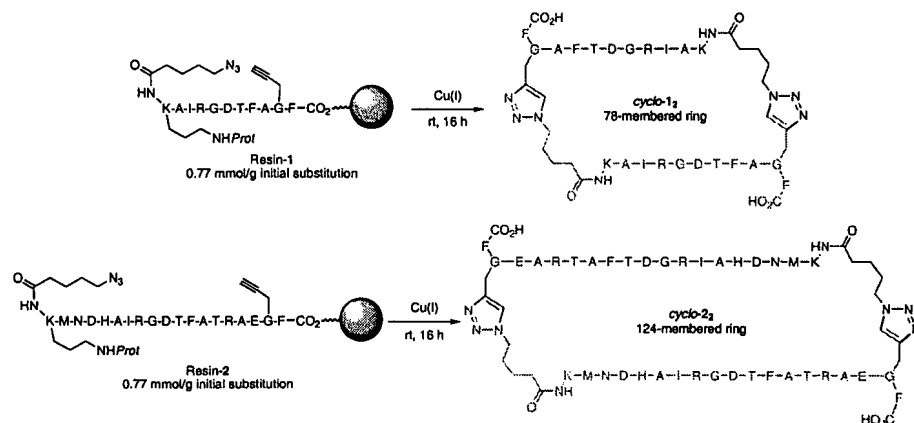
Ref. 46

Scheme 3. Cyclodimerization of Peptides on Solid Support.
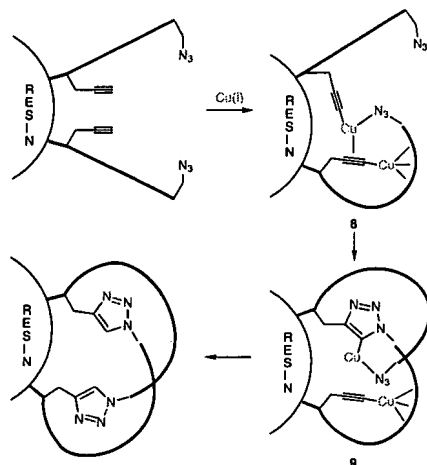
Ref. 46

Scheme 4. Possible Intermediates in the Cyclodimerization of Peptides on Solid Support.

Resin-1 and Resin-2, contained L-propargylglycine as the second residue on one end, and the other end of each chain was capped with 5-azidopentanoic acid. Exposure of each of these resins to Cu(I) for 16 h at room temperature resulted in the consumption of all of the azide groups and the complete disappearance of the linear oligopeptides. However, the single dominant species in each case was the cyclic dimer, cyclo-1_2 and cyclo-2_2, rather than the monomer of the desired sequence; isolated yields were in the 80–90% range, which is excellent for peptides of this size.

An extensive series of experiments established that the cyclodimerization process was dependent on the density of peptides on the resin and did not occur as efficiently off the resin.[46] The cyclodimerization was inhibited by excess copper(I), addition of a copper-binding chelating ligand that normally promotes other AAC reactions, or added phenylacetylene (but not added azide). **Scheme 4** illustrates several possible explanations of these observations. Since the ligand-free system showed a rate order in PhC≡CH of between 1 and 2, a second-order pathway in alkyne is possible, with one alkyne playing a structural role. If, for example, one copper acetylide of associated chains is π-complexed to the other, as shown in structure 8, the reactivities of the two acetylides
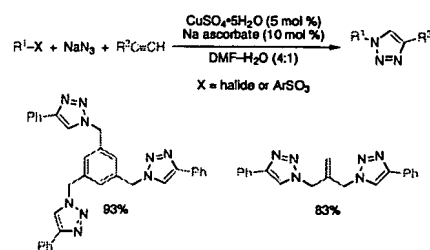
Ref. 48–51

eq 2

toward the azide will be different. Alternatively, interaction of the azide at the terminus with the copper acetylide at its base should be entropically less favorable than interaction with another copper acetylide fragment, as also represented in structure 8. Furthermore, once the first cycloaddition event takes place (leading to 9), the organometallic fragment may bind and direct the second azide to engage the remaining acetylide and complete the macrocyclization. Angell and Burgess proposed a similar hypothesis to explain their observation of a strong preference for the formation of cyclic dimers from small peptides in solution.[47]

2.4. One-Pot CuAAC Procedure

Although organic azides are generally safe compounds, those of low molecular weight can be unstable and, therefore, difficult to handle. This is especially true for small molecules with several azide functionalities that would be of much interest for the generation of polyvalent structures. In these cases, CuAAC can be performed as a one-pot two-step sequence, whereby an in situ generated organic azide is immediately consumed in a reaction with a copper acetylide. Alkyl azides can be readily obtained from the corresponding halides or arylsulfonates by reaction with sodium azide (eq 2).[48–51] Residual NaN₃, even when used in excess in the alkyl azide forming step, does not interfere with the subsequent cycloaddition reaction.

Similarly, aryl and vinyl azides are available in one step from aryl halides via a copper-catalyzed reaction with sodium azide in the presence of a catalytic amount of L-proline (eq 3).[48] Using this approach, a range of 1,4-disubstituted 1,2,3-triazoles were prepared in excellent yields.[49–51] The same reaction sequence can be

performed at elevated temperature under microwave irradiation, reducing the reaction time to 10–30 minutes.[36]

2.5. Reactions of Sulfonyl Azides

The reaction of sulfonyl azides with terminal alkynes under copper catalysis is an interesting exception. Depending on the conditions and reagents, it can result in the formation of different products (**Scheme 5**). As reported by Chang and co-workers, *N*-sulfonyl azides are converted to *N*-sulfonyl amidines when the reaction is conducted in the presence of amines.[52] Under aqueous conditions, *N*-acylsulfonamides are the major products.[53,54]

Although the catalysis initially appears to follow the same pathway as in other CuAAC reactions of azides, the cuprated triazole intermediate **10** (**Scheme 6**)—the *N*-sulfonylated analogue of **7** in Scheme 2—can undergo a ring–chain isomerization to form the cuprated diazoimine **11** which, upon the loss of a molecule of dinitrogen, furnishes the *N*-sulfonylketenimine **12**.[55] Alternatively, copper(I) alkynamide **13** can be generated with a concomitant elimination of dinitrogen and, after protonation, would also generate the same reactive *N*-sulfonylketenimine species **12**. In addition to amines and water, **12** can be trapped with imines, furnishing *N*-sulfonylazetidinimines.[55] However, when the reaction is performed in chloroform in the presence of 2,6-lutidine, *N*-sulfonyltriazoles are obtained in good yields.[56]

3. Selected Applications of CuAAC
3.1. Synthesis of Small-Molecule Screening Libraries

The CuAAC process performs well in most common laboratory solvents and usually does not require protection from oxygen and water (in fact, aqueous solvents are commonly used and, in many cases, result in cleaner isolated products), making it an ideal tool for the synthesis of libraries for initial screening as well as for structure–activity profiling. The lack of byproducts and high conversions often allow screening of the reaction products without further purification. When necessary, traces of copper and byproducts can be removed by solid-phase extraction utilizing a metal-scavenging resin or by simple filtration through a plug of silica.

3.1.1. Inhibitors of Fucosyl Transferases

Sialyl Lewis x (sLe^x) and sialyl Lewis a (sLe^a) of the cell-surface glycoproteins and glycolipids play a central role in cell–cell interactions and cell migration in physiological and pathological processes such as fertilization, embryogenesis, inflammation, and cancer metastasis.[57] The terminal step in the biosynthetic pathway of these fucose-containing saccharides is the transfer of L-fucose from guanosine diphosphate β-L-fucose (GDP-fucose) to the corresponding glycoconjugate acceptor by action of fucosyl transferases (FucTs).[58] The selective inhibition of FucTs is an attractive treatment for pathologies associated with the aforementioned glycoconjugates. However, the low substrate (GDP-fucose and sialyl *N*-acetyllactosamine) affinity, combined with the lack of enzyme structural data, make the rational development of potent inhibitors for FucTs difficult. In a study reported by Lee et al., CuAAC was used to link 85 azides to the GDP-derived alkyne **14** with excellent yields (**eq 4**).[59] The absence of offensive byproducts allowed the direct screening of the library, and resulted in the identification of a nanomolar inhibitor, **15**. Testing purified **15** against several glycosyltransferases and nucleotide-binding enzymes revealed that it was up till then the most potent and selective inhibitor of human α-1,3-fucosyltransferase VI .

eq 3

Ref. 48–51

Ref. 52–56
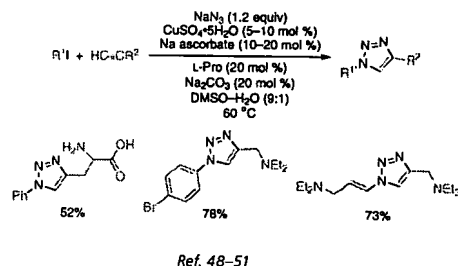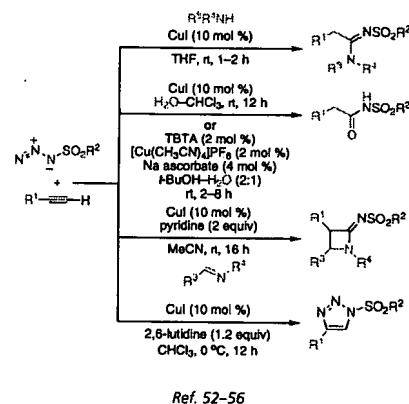
Scheme 5. The Dependence of the Outcome of the Reaction of Sulfonyl Azides with Alkynes on the Reaction Conditions.

Ref. 55,56
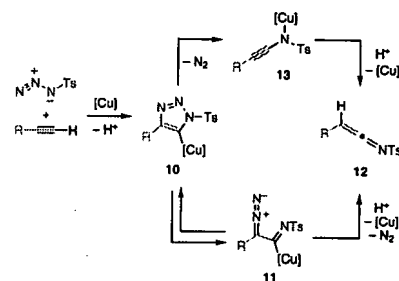
Scheme 6. Postulated Pathways That Lead to a Common Ketenimine Intermediate in the CuAAC Reaction of Sulfonyl Azides with Alkynes.

Ref. 59

eq 4

For R' = (17)

	HIV-1 PR	V82F	G48V	V82A
K_i (nM) =	4	13	9.7	30

Ref. 60,61

eq 5

18, K_i = 98 ± 2 nM

19, K_i = 23 ± 4 nM

20, K_i = 8 ± 0.5 nM
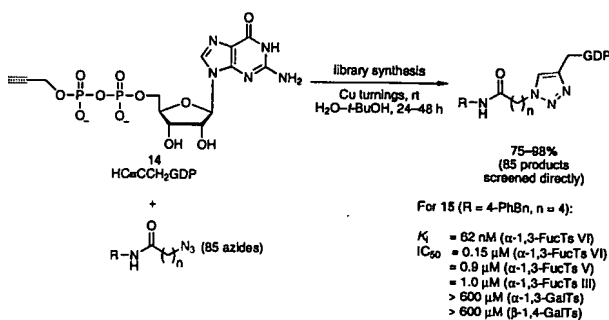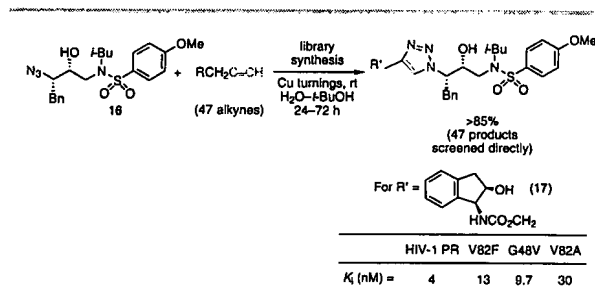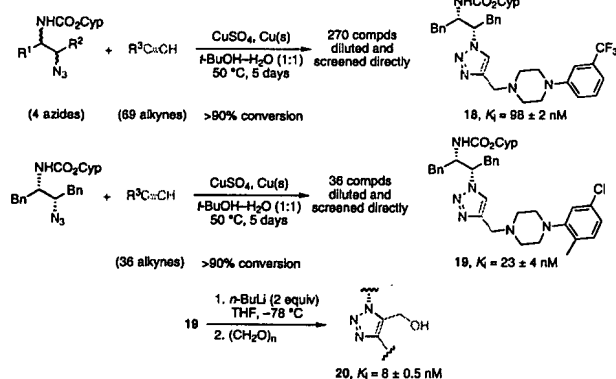
Ref. 62

Scheme 7. Novel Series of Inhibitors of HIV-1 Protease.
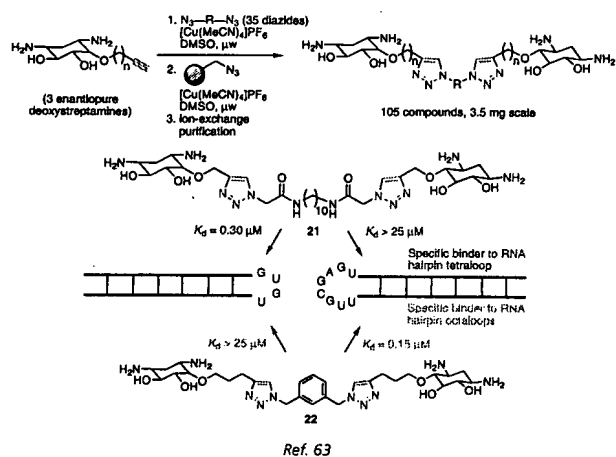
Ref. 63

eq 6
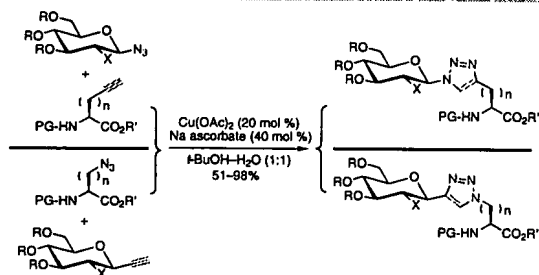
Ref. 64

Scheme 8. Synthesis of Triazole-Linked Glycopeptides.

3.1.2. HIV-1 Protease Inhibitors

HIV-1 protease has been recognized as an important target for the inhibition of viral replication. Although seven inhibitors have been approved by the FDA since 1995, and a number more are currently undergoing clinical evaluation, their success has been undermined by the alarmingly rapid mutation of the virus. In one study, a focused library of about 50 compounds was prepared utilizing CuAAC as the last assembly step.[60] The azide-decorated scaffold 16, inspired by the structure of Amprenavir, and a selection of 47 alkynes, were united through a 1,2,3-triazole unit by simply shaking the reactants with a piece of copper turning for 72 hours. After dilution with water, the compounds were screened, without purification, against the wild type HIV-1 protease and three mutants (G48V, V82F, and V82A). Several hits were identified, and the two most active compounds were resynthesized, and their IC_{50} and K_i values were determined (eq 5; only one active, 17, is shown). Analysis of the crystal structures of the HIV-1 protease with bound inhibitor 17 confirmed that the triazole ring acted as an amide-bond surrogate, retaining all hydrogen bonds in the active site of the enzyme.[61]

Another recent study disclosed a novel series of potent HIV-1 protease inhibitors that have been developed.[62] CuAAC was used to unite a focused library of azide-containing fragments with a diverse array of functionalized alkyne-containing building blocks (Scheme 7, top). After the direct screening of the crude reaction products, a lead structure, 18, with K_i = 98 nM was identified. Optimization of both azide and alkyne fragments proved equally facile (e.g., 19; K_i = 23 nM). Further functionalization of the triazole at C-5 gave a series of compounds with increased activity, exhibiting K_i values as low as 8 nM (20).

3.1.3. Size-Specific Ligands for mRNA Hairpin Loops

The development of ligands that target mRNAs coding for proteins that lack small-molecule binding sites could be a promising approach to drug discovery. To this end, Hergenrother's group reported an approach utilizing CuAAC for the synthesis of a screening library of mRNA hairpin loop binders.[63] A collection of 105 deoxystreptamine dimers from three alkynylated derivatives of enantiopure deoxystreptamines and thirty-five diazides was prepared (eq 6). Under microwave heating, the reactions proceeded almost to completion in 40 seconds. After treating the reaction mixture with a resin-supported azide to scavenge unreacted alkyne, the bistriazole products of 91% average purity were isolated and screened for size-specific mRNA hairpin loop binding. Two hits, 21 and 22, which exhibit complementary specificity (for mRNA tetraloops and octaloops, respectively), were identified.

3.2. Synthesis of Neoglycoconjugates

The importance of glycoconjugates as mediators of complex cellular events has inspired the development of new methodologies for the construction of glycoproteins with well-defined, homogeneous glycoforms. One of the difficulties inherent in the synthesis of N- and O-linked glycopeptides is the sensitivity of glycosidic linkages toward chemical and enzymatic hydrolysis. This problem could potentially be solved by introducing a chemically and metabolically stable isosteric linkage such as 1,2,3-triazole. Thus, two high-yielding syntheses of triazole-linked glycopeptides have been achieved by Rutjes and co-workers.[64] CuAAC chemistry demonstrated equal efficiency for the coupling of either azidoglycosides with acetylenic oligopeptides or acetylenic glycosides with azide-containing peptides (Scheme 8).

Many cell-surface recognition events, which result in cellular adhesion, inflammation, and immune surveillance are mediated by complex carbohydrate interactions. It is therefore not surprising

that the development of synthetic architectures that bear multiple carbohydrate units has received much attention. CuAAC— utilizing organic-soluble catalysts [Cu(Ph₃P)₃]Br and (EtO)₃P•CuI in combination with Et(i-Pr)₂N—have been employed by Santoyo-Gonzalez and co-workers to synthesize a number of poly- (di- to hepta-) mannosylated ligands (**Figure 3**).[32] Under microwave irradiation, the reactions were generally complete in less than 30 minutes, and polyvalent products were isolated in high yields. More recently, C-glycoside clusters,[65] neoglycopolymers,[66] and virus-glycopolymer conjugates[67] have been reported utilizing CuAAC alone or in combination with atom-transfer polymerization techniques.

3.3. Modification and Biological Profiling of Natural Products

Many bioactive natural products have limited therapeutic potential because of the narrow activity–toxicity window (the minimum therapeutically useful concentration and the concentration at which side effects make treatment impractical). Therefore, the modification of potentially useful bioactive natural products is a viable approach towards improving their therapeutic index. Ideally, such a modification should be accomplished on the molecule itself to avoid protection–deprotection steps. In reality, most chemical transformations are not compatible with a range of functional groups present in the parent compound. The high selectivity and fidelity of the CuAAC make it a good candidate for the last-step derivatization of complex bioactive molecules. This approach was demonstrated by Lin and Walsh, who utilized CuAAC for the preparation of an analogue library of the glycopeptide antibiotic tyrocidine,[68] a nonribosomal peptide which is believed to target the bacterial lipid bilayer. Although a potent antibiotic, it can cause hemolysis at concentrations close to MIC. First, thirteen head-to-tail cyclic tyrocidine derivatives containing one to three propargylglycines were generated from linear N-acetylcysteamine peptides by the action of a thioesterase domain excised from tyrocidine synthetase (**Scheme 9**). The resulting cyclic peptides were then conjugated to twenty-one azido sugars at less than 1 mM concentration, resulting in a library of 247 isolated compounds. The two most active of these, **23** and **24**, identified by antibacterial and hemolysis assays, were purified and further profiled, revealing a 6-fold improvement in the therapeutic index when compared to wild-type tyrocidine. The toxicity of different reagents that can be used in the CuAAC step was also examined: neither ascorbate, Cu(II), nor TBTA caused a hemolytic reaction, thus allowing the direct assay of the analogue library.

The biological activity of natural products is often modulated by their glycosylation, and recent studies suggest that novel therapeutics could be generated by altering the glycosylation patterns of their secondary metabolites. To study the effects of such glycosylation, Thorson and co-workers took advantage of CuAAC in a glycorandomization study performed on vancomycin (eq 7).[69–71] In the first step, a number of sugar precursors bearing reactive handles were covalently linked to the heptapeptide aglycon regio- and stereospecifically to furnish a library of monoglycosylated vancomycin analogues. This one-pot, three-step process was mediated by three enzymes: a sugar kinase, a nucleotidyltransferase, and a glycosyltransferase. Monoglycosylated derivative **25**, containing an azide handle, was further diversified using CuAAC with a selection of alkynes to create a small library of vancomycin analogues. Antibacterial screens of the resulting library identified carboxylic acid derivative **26** as having twice the activity of vancomycin against both S. aureus and E. faecium.
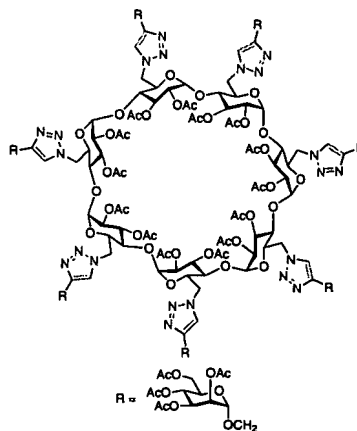
Ref. 32

Figure 3. Heptamannosylated Cyclodextrin Derivative Synthesized by CuAAC in One Step.
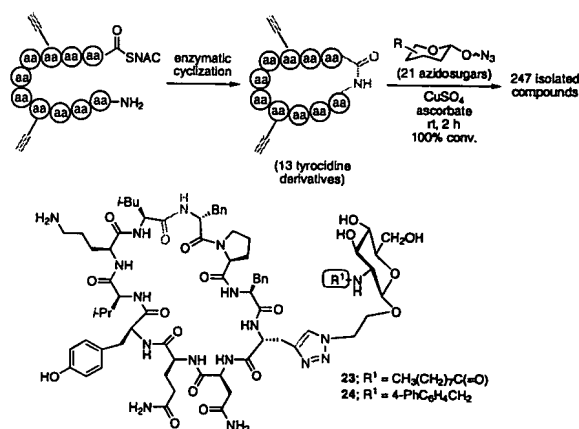
Ref. 68

Scheme 9. Chemoenzymatic Functionalization of the Antibiotic Tyrocidine.

Ref. 69–71

eq 7
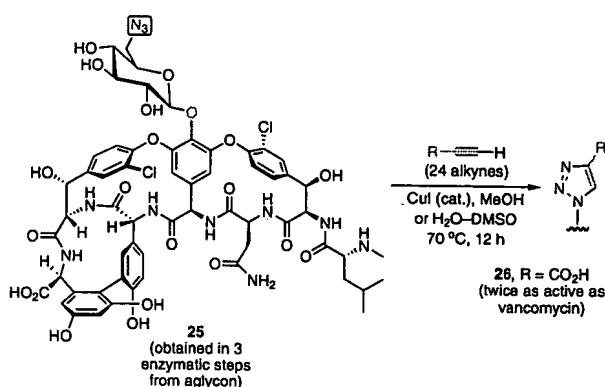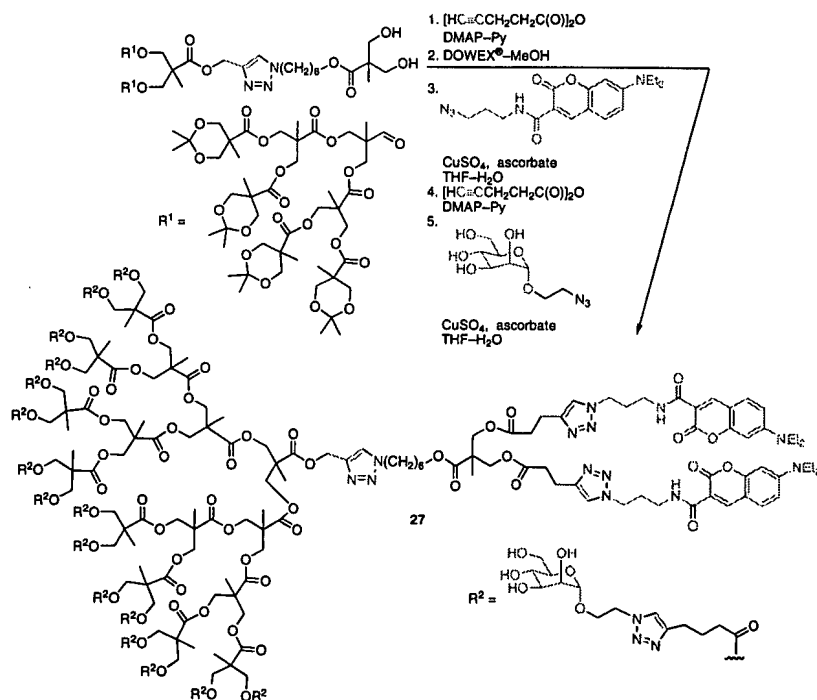
Scheme 10. CuAAC-Enabled Synthesis of a Bifunctional Dendrimer.

3.4. Bioconjugation: Following the Dynamics of Proteomes

Incorporation of amino acids containing small bioorthogonal groups into the proteome of an organism, either metabolically (i.e., by "tricking" the organism's own biosynthetic machinery to co-translationally introduce unnatural amino acids into its proteins) or through site-directed mutagenesis,[72,73] allows the tracking of proteome dynamics in response to external stimuli. The advantages of the azide functionality for selective chemical ligation have been demonstrated in many studies involving the modified Staudinger ligation.[74–76] CuAAC has thus emerged as a complementary strategy, boasting high reaction rates and exquisite selectivity.[40,77,78] This methodology was used by Tirrell and co-workers to selectively label azide- or alkyne-containing amino acids, such as azidohomoalanine, homopropargylglycine, or ethynylphenylalanine, which were co-translationally incorporated into the newly synthesized proteins of *E. coli.*[78,79] In the last step, the exposed azide or alkyne residues were conjugated with either a biotin label or a "pro-fluorescent" coumarin derivative, which is activated once the azide is converted to the 1,2,3-triazole heterocycle.[80] The ligand TBTA was essential for the functionalization step. Since the pro-fluorescent coumarin labeling reagent contributes only weakly to background signals, the labeled protein could be directly observed without the need for washing, Western blotting, or secondary labeling steps. The approach has been recently extended to the study of proteomes in mammalian cells.[81] This method complements the gene-encoded labeling approaches[77,82,83] for investigating protein structures and functions in vivo.

3.5. Synthesis of Functional Dendrimers

As highly ordered, regularly branched, globular macromolecules of defined structure, dendrimers are ideal scaffolds for creating bioactive nanomaterials. The high fidelity of CuAAC, its tolerance of most functional groups, and simple product isolation have enabled the efficient construction of dendrimers utilizing procedures that involved little more than mixing stoichiometric quantities of reactants, stirring, and isolating the dendrimeric products.[34,84–87] Purification was not required up to the third generation.[88] The same features of CuAAC that allowed the synthesis of novel dendrimers have also been exploited for chain-end functionalization of dendrimers and hyperbranched polymers.[89–93] An example is the covalent attachment of 3'-azido-3'-deoxythymidine to the dodeca-alkyne dendritic core, providing the nucleoside-terminated dendrimer in 94% isolated yield at room temperature in aqueous solution.[33] The ability to covalently label dendritic scaffolds with nearly any functional group allows the preparation of complex dendritic libraries in multigram quantities under very mild conditions. For example, CuAAC enabled a high-yield synthesis of a chemically heterogeneous dendrimer 27 containing 16 α-D-mannose units and two coumarin-derived fluorescent chromophores (**Scheme 10**).[94] The performance of this bifunctional dendrimer was evaluated in a standard hemagglutination assay using the mannose-binding protein concanavalin A and rabbit red blood cells. Dendrimer 27 exhibited a 240-fold greater potency than monomeric mannose, which translates into a 15-fold increase in activity per unit. Syntheses of peptidodendrimers,[95] unprotected glycodendrimers,[93,96] and redox-active dendrimers for sensor applications[97] have also been described.

3.6. Polyvalent Display with Viruses

Viruses present another opportunity for the polyvalent presentation of functional molecules.[98–100] A number of plant viruses of different sizes have been structurally characterized, and many of these are available in large quantities. They are

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stable to a variety of chemical agents, and their capsid proteins can be selectively modified using site-directed mutagenesis, allowing facile introduction of azide or alkyne handles. For example, the cow pea mosaic virus (CPMV) has been derivatized with single carbohydrate compounds producing a dendrimer-like display with polyvalent lectin-binding properties (eq 8);[101] with PEG to give well-controlled loadings of polymer on the outer surface of the coat protein assembly;[102] and, more recently, with glycopolymers.[67]

3.7. Functionalization of Self-Assembled Monolayers

The chemical modification of surfaces precoated with self-assembled monolayers (SAMs) of alkanethiols facilitates studies aimed at the fundamental understanding and rational control of electron-transfer events at the electrochemical interface.[103] As reported by Collman et al. and others, cycloaddition chemistry allows the facile introduction of redox-active ferrocene derivatives onto SAM-coated electrode surfaces.[104,105] In one example, monolayers containing azide terminal groups were formed by coadsorption of azidoundecanethiols with decanethiols to gold-coated silicon wafers. Ethynylferrocene was coupled to the monolayers using 1 mol % of copper(II) sulfate and 15 mol % of sodium ascorbate, and the progress of the reaction was followed by the disappearance of azide absorption in infrared spectra. The density of coverage was quantified using cyclic voltammetry. In another report, the copper(I) catalyst was electrochemically formed on the surface of independently addressed microelectrodes (separated by 10 μm). Application of a –300-mV potential to the targeted electrodes resulted in the instantaneous formation of Cu(I) from the Cu(II) salt, thereby providing a catalytically active species that effected cycloaddition of ethynylferrocene to the azide-containing SAM.[106]

4. Summary and Outlook

The rapid emergence of wide-ranging applications of the copper-catalyzed azide–alkyne cycloaddition (CuAAC) in a few short years since its discovery underscores the immense need for other reliable, chemically orthogonal transformations for establishing covalent connections between diverse building blocks. Equally important, the revived interest in the well-known, yet notably underutilized, class of heterocycles, 1,2,3-triazoles, has served as an impetus for the development of other selective methods leading to this functionality. Thus, ruthenium(II) complexes were recently reported to catalyze the cycloaddition of both terminal and internal alkynes, resulting in the formation of 1,4-disubstituted- and 1,4,5-trisubstituted-1,2,3-triazoles, isomers that are not obtained via CuAAC.[107] Noncatalytic variants of the cycloaddition, which rely on the activation of the alkyne component by means of electron-withdrawing substituents or strain have also been revisited. For example, a strain-promoted cycloaddition, which takes advantage of the reactive nature of cyclooctyne and, therefore, does not require a catalyst, was used to label N-azidoacetyl neuramic acid residues on cell surfaces.[108,109] This application also highlights a current limitation of CuAAC: free copper ions are cytotoxic and may cause degradation of proteins and nucleic acids in vitro. Although Cu(I)-stabilizing ligands provide an acceptable solution in many cases, the development of new catalysts and ligands that prevent the leaching of copper ions into the reaction medium, yet do not impair the catalytic activity of copper, will significantly expand the reach of this already widely utilized transformation, and might enable the selective manipulation of living systems at the molecular level—a powerful, yet challenging strategy.[76]
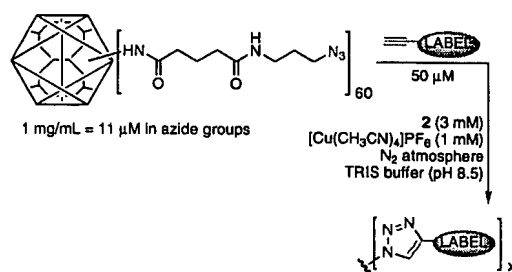
1 mg/mL = 11 μM in azide groups

50 μM

2 (3 mM)
[Cu(CH₃CN)₄]PF₆ (1 mM)
N₂ atmosphere
TRIS buffer (pH 8.5)

Ref. 42,98–102

eq 8

6. References and Notes

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About the Authors

Peng Wu was born in Yaan, China. After receiving his B.S. degree in chemistry from Peking University in 1999, he joined Professor Kenneth G. Caulton's research group at Indiana University, Bloomington, where he studied ruthenium and copper pincer complexes. In 2001, he entered the graduate program at The Scripps Research Institute and, in 2005, received the Ph.D. degree under the guidance of Professors K. Barry Sharpless and Valery V. Fokin. While at Scripps, he developed a general method for the synthesis and functionalization of dendrimers using the newly discovered copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction. From September 2004 to early 2005, he was a visiting student in the laboratory of Professor Craig J. Hawker at IBM Almaden Research Center and at the University of California, Santa Barbara. Currently, Dr. Wu is an HHMI postdoctoral fellow in the group of Professor Carolyn R. Bertozzi at the University of California, Berkeley.

Valery V. Fokin was born in 1971 in Nizhny Novgorod, Russia. He received his *Diploma* in chemistry in 1993 from the University of Nizhny Novgorod, and his Ph.D. degree in 1998 from the University of Southern California, Los Angeles, where he worked under the direction of Professor Nicos A. Petasis. He then joined the group of Professor K. Barry Sharpless at The Scripps Research Institute in La Jolla, California, as a postdoctoral fellow, where he studied catalytic oxidations of olefins. In 2000, he was appointed Assistant and, in 2006, Associate Professor in the Chemistry Department at Scripps. His research is centered on understanding chemical reactivity and its applications in chemistry, biology, and materials science.◓

GENERAL REFERENCE

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Peng Wu and Valery V. Fokin

Rapid Discovery and Structure—Activity Profiling of Novel Inhibitors of Human Immunodeficiency Virus Type 1 Protease Enabled by the Copper(I)-Catalyzed Synthesis of 1,2,3-Triazoles and Their Further Functionalization

Matthew Whiting,[†] Jonathan C. Tripp,[†] Ying-Chuan Lin,[‡] William Lindstrom,[‡] Arthur J. Olson,[‡] John H. Elder,[‡] K. Barry Sharpless,[†] and Valery V. Fokin*,[†]

Departments of Chemistry and Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Building from the results of a computational screen of a range of triazole-containing compounds for binding efficiency to human immunodeficiency virus type 1 protease (HIV-1-Pr), a novel series of potent inhibitors has been developed. The copper(I)-catalyzed azide—alkyne cycloaddition (CuAAC), which provides ready access to 1,4-disubstituted-1,2,3-triazoles, was used to unite a focused library of azide-containing fragments with a diverse array of functionalized alkyne-containing building blocks. In combination with direct screening of the crude reaction products, this method led to the rapid identification of a lead structure and readily enabled optimization of both azide and alkyne fragments. Replacement of the triazole with a range of alternative linkers led to greatly reduced protease inhibition; however, further functionalization of the triazoles at the 5-position gave a series of compounds with increased activity, exhibiting K_i values as low as 8 nM.

Introduction

The global AIDS epidemic has claimed the lives of more than 20 million people since 1981. Another 40 million are now living with HIV, and most of these are likely to develop AIDS over the course of the next decade. Despite the various treatment protocols available, including the mainstream, highly active antiretroviral therapy (HAART),[1] the number of people infected with HIV continues to rise. The most recent UNAIDS/WHO estimates show that, in 2004 alone, 4.9 million people were newly infected with HIV.[2] HIV-1 protease[3] has been recognized as an important target for inhibition of viral replication. Although ten inhibitors have been approved by the FDA since 1995, and a number more are currently undergoing clinical evaluation, their success has been undermined by rapid mutation of the virus.[4] The alarming rate at which strains of HIV-1 that are resistant to the currently available drugs and their combinations are emerging, underscores the urgent need for new, broad-spectrum protease inhibitors, which are effective against the new mutants as well as the wild-type virus.

Herein we report the use of several click chemistry[5] techniques to aid the rapid identification and optimization of a novel series of HIV-1-Pr inhibitors. The copper(I)-catalyzed azide—alkyne cycloaddition (CuAAC)[6] has cemented its position at the heart of click chemistry due to its extremely broad scope, the orthogonality of the azide and alkyne functionalities to a diverse range of functional groups and reaction conditions, and the unique properties of the triazole products. In combination with direct screening of the crude reaction mixtures, it has proven to be a powerful approach for the rapid discovery of novel, biologically active molecules.[7] In this fashion, focused libraries of alkyne- and azide-bearing fragments, which themselves exhibited only minimal inhibitory activities, were combined under copper(I) catalysis, and the crude reaction products were directly screened for protease inhibition. Products exhibiting the highest inhibition values were then independently synthesized to confirm their activity and used as the basis for further fragment optimization. After identification of the azide- and alkyne-containing fragments resulting in the most potent inhibitors, the triazole linker itself was either replaced or further functionalized to give a series of highly active compounds with K_i values against HIV-1 protease as low as 8 nM.

Results and Discussion

An initial lead structure was chosen from an in silico screen of an array of triazole-containing candidate structures. Thus, computational docking of an initial set of compounds into the active site of HIV-1-Pr, by use of the AutoDock program,[8] enabled calculation of their relative binding energies. Taking into account the computed binding energies as well as the potential for rapid diversification of the lead structure, compound 1 was chosen for further exploration. This structure had a relatively low computed binding energy (within the range of AutoDock standard deviation[8] from that of Amprenavir) and synthetically could readily be obtained from a core α-amino azide unit, a carbonyl-based capping group, and an alkyne (Scheme 1). Hence a small group of amino azide cores could be rapidly derivatized with activated carbonyl reagents followed by CuAAC with a library of alkynes to give a highly diverse array of potential inhibitors.

Scheme 1. Compound 1: Its General Structure and Proposed Synthesis from an Amino Azide Core, an Activated Carbonyl Compound, and a Terminal Alkyne

Computed binding energy = -15.7 kcal/mol
c.f. Amprenavir = -16.5 kcal/mol

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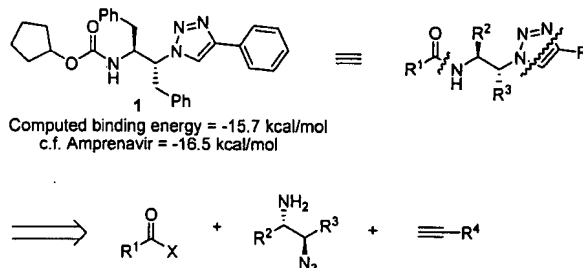
* To whom correspondence should be addressed: tel (+1) 858-784-7515; fax (+1) 858-784-7562; e-mail fokin@scripps.edu.
† Department of Chemistry.
‡ Department of Molecular Biology.

Scheme 2. Planned Synthesis of α-Amino Azide Cores[a]

[a] PG, protecting group.
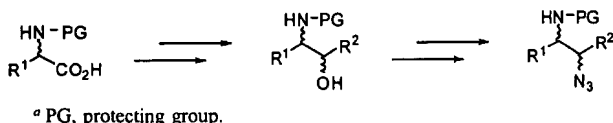
Scheme 3. Synthesis of Syn α-Amino Azides
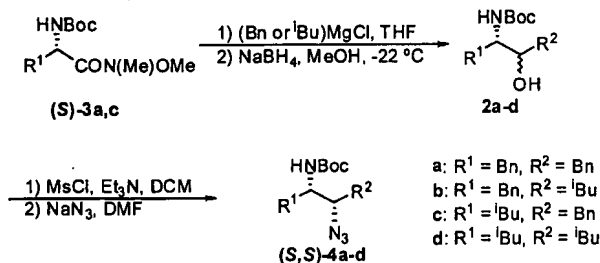
compd	crude ratio anti:syn-2[a]	isolated yield % anti-2[b]	syn-4[c]
a	90:10	61	58
b	83:17	51	38
c	88:12	64	59
d	>95:5	44	41

[a] Determined from the crude ¹H NMR spectra. [b] Isolated yield of the isomerically pure anti amino alcohol over two steps from the corresponding Weinreb amide. [c] Isolated yield of the isomerically pure syn amino azide over two steps from the corresponding anti amino alcohol.

Synthetically, the α-amino azides were the most challenging fragments to obtain. Both enantiomers of both the syn and anti isomers of the amino azides were desired in order to fully probe the structural space. Although a number of selective routes to these compounds can be envisaged (for example, asymmetric functionalization of the corresponding alkenes),[9] the selective manipulation of α-amino acid derivatives[10] was the most convenient for our purposes, enabling synthesis of both diastereomers incorporating a range of substituents. Thus, suitable amino acid derivatives were selectively converted to the corresponding amino alcohols, which, after activation, readily gave the required amino azides (Scheme 2).

For the initial screening process we opted to make the compounds with R¹ and R² = ⁱBu or Bn, the N-Boc protecting group was chosen due to its ease of removal. Starting from the naturally occurring enantiomers of isoleucine and phenylalanine, all eight regio- and diastereoisomers of the amino azides were synthesized on approximately 1 g scale. Representative enantiomeric compounds were also synthesized starting from the unnatural amino acid derivatives.

Despite a large body of literature on the selective synthesis of α-amino alcohols[10,11] the majority of the compounds we required had not been previously described in the literature. Their syntheses, based on modified literature procedures, are described below. The anti amino alcohols (*S,R*)-2a—d were readily obtained by chelation-controlled reduction of the corresponding α-amino ketones[12] which were themselves obtained by the addition of benzyl or isobutylmagnesium chloride to the Weinreb amide derivative of N-Boc-L-phenylalanine (*S*)-3a or N-Boc-L-isoleucine (*S*)-3c. Mesylation under standard conditions followed by displacement of the mesylate group with sodium azide gave the corresponding syn amino azides (*S,S*)-4a—d in average to good yield (Scheme 3).[13]

The syn amino alcohols (*S,S*)-2a—d were obtained with reasonable selectivity by chelation-controlled addition of either isobutylmagnesium chloride or the cuprate reagent derived from benzylmagnesium chloride and copper bromide—dimethyl sul-

Scheme 4. Synthesis of Anti α-Amino Azide
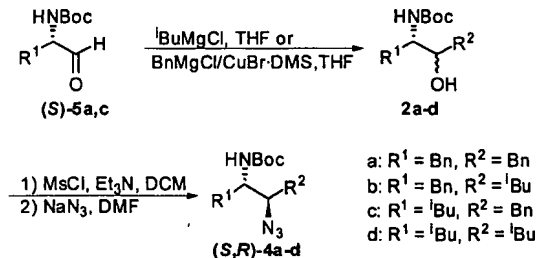
compd	crude ratio. syn:anti -2[a]	isolated yield % syn-2[b]	anti-4[c]
a	80:20	47	67
b	88:12	47	42
c	80:20	55	71
d	-[d]	63	50

[a] Determined from the crude ¹H NMR spectra. [b] Isolated yield of the isomerically pure syn amino alcohol. [c] Isolated yield of the isomerically pure anti amino azide over two steps from the corresponding syn amino alcohol. [d] Not determined.

Scheme 5. Synthesis of α-Amino Azides from D-Phenylalanine Derivatives
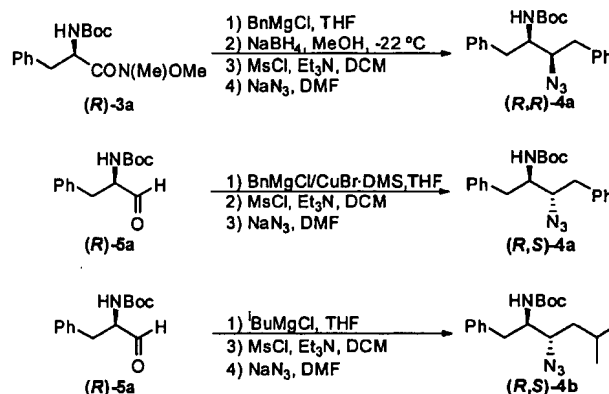
fide complex[14] to the N-Boc-protected amino aldehydes, N-Boc-L-phenylalaninal (*S*)-5a or N-Boc-L-isoleucinal (*S*)-5c.[15] Interestingly, use of either benzylmagnesium chloride or the isobutyl cuprate reagent was not successful. Mesylation of the product alcohols (*S,S*)-2a—d under standard conditions followed by displacement of the mesylate group with sodium azide furnished the corresponding anti amino azides (*S,R*)-4a—d in acceptable yield (Scheme 4).

Three enantiomers of 4 were also synthesized, both to allow determination of their biological activities and to confirm the enantiomeric purity of our compounds by chiral HPLC analysis. The syn and anti compounds with R¹ = R² = Bn, (*R,R*)-4a and (*R,S*)-4a, and also the anti compound with R¹ = Bn and R² = ⁱBu, (*R,S*)-4b, were synthesized following the routes described above, starting from the unnatural amino acid derivatives N-Boc-D-phenylalanine N-methoxy-N-methylamide (*R*)-3a, and N-Boc-D-phenylalaninal (*R*)-5a (Scheme 5). Chiral HPLC analysis[16] of the three pairs of enantiomers confirmed their enantiomeric purity (ee > 98%), confirming that no racemization had occurred during any of our three synthetic routes.

The 11 N-Boc-protected amino azides 4 were then converted to their corresponding cyclopentyl carbamates 6 for initial fragment screening. Thus, cleavage of the N-Boc protecting groups with trifluoroacetic acid (TFA) in dichloromethane (DCM) followed by reaction of the free amines with cyclopentyl chloroformate under biphasic conditions provided the desired

Scheme 6. Synthesis of Cyclopentyl Carbamates (6)

(S,S)-4a-d
(S,R)-4a-d
(R,R)-4a
(R,S)-4a-b

(S,S)-6a-d
(S,R)-6a-d   } 68-93 %
(R,R)-6a
(R,S)-6a-b

a: R¹ = Bn, R² = Bn
b: R¹ = Bn, R² = ¹Bu
c: R¹ = ¹Bu, R² = Bn
d: R¹ = ¹Bu, R² = ¹Bu
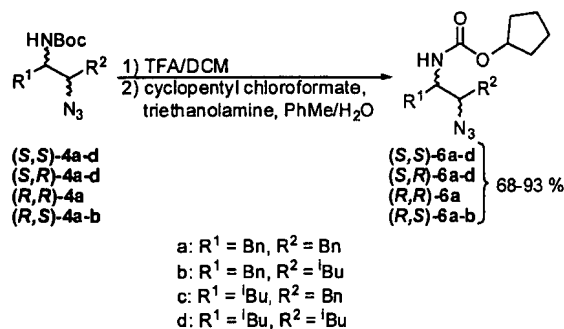
Scheme 7. Synthesis of a Screening Library

6 → 7
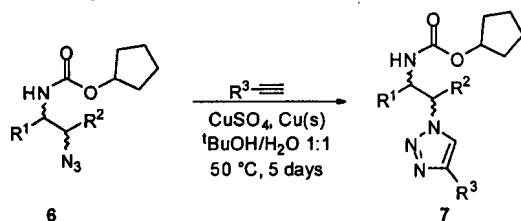
products (6) in high yield and over 95% purity (as determined by liquid chromatographic—mass spectrometric analysis, LC-MS) after a simple aqueous workup (Scheme 6).

The amino azide derivatives (6) were then subjected to CuAAC with a selection of 69 terminal alkynes incorporating a diverse range of functionalities,[16] producing a library of triazoles with general structure 7 (Scheme 7). A solution of each azide core in ¹BuOH was added individually to a separate array of reaction tubes, each containing a solution of a single alkyne in ¹BuOH. An aqueous solution of CuSO₄ and a small piece of copper wire were added to generate a constant source of Cu(I). The tubes were sealed and shaken at 50 °C for 5 days, after which time LC-MS analysis indicated complete conversion of the starting azide to the corresponding triazole product in the vast majority of cases. The crude reaction mixtures were diluted

to 5 μM into 96-well plates and assayed directly for their inhibitory activity against HIV-1-Pr.[17] Five of these compounds, (S,S)-7a—e (Figure 1), were found to give over 20% inhibition (Figure 1). All of the hits contained an S,S-configured syn amino azide fragment and an alkyne fragment based on the propargylpiperazine motif.

Synthesis and isolation of the two most potent compounds, (S,S)-7d and (S,S)-7e, proved these were genuine hits with K_i values of 98 ± 2 nM and 86 ± 9 nM, respectively (Scheme 8).

After identification of the initial hits, two focused libraries were synthesized to rapidly optimize both the alkyne and the azide-containing fragments. Optimization of the former was achieved by reaction of the dibenzyl syn amino azide (S,S)-4a with an array of 36 propargylpiperazine and -piperidine derivatives in the same manner as described above to give an array of 36 triazoles (S,S)-11.[16] A number of new hits were identified; all the most potent of which contained an arylpiperazine moiety (selected examples are shown in Table 1). The parent 4-phenylpiperazine [Table 1, compound (S,S)-11a] maintained good activity; however, alteration of this structural motif gave greatly reduced inhibition [(S,S)-11b—e]. Incorporation of 4-heteroarylpiperazines also resulted in greatly reduced activity [(S,S)-11f]. Variation of the degree and nature of substitution on the phenyl ring was well tolerated [(S,S)-11g—k], with 2,5-disubstitution tending to give the more potent compounds [(S,S)-11j,k]. Again, the most potent compounds were individually synthesized and isolated, and their K_i values were determined (Table 1).

The azide-containing fragment was profiled by replacing the cyclopentyl carbamate group that had been used initially with a range of alternative carbonyl-based caps. Thus, free amine (S,S)-12a was combined with an array of acyl chlorides, isocyanates, chloroformates, and O-succinamidyl carbonates under biphasic conditions to give a selection of 23 amides, ureas, and carbamates [(S,S)-13] in high yield and again over 95% purity (as determined by LC-MS analysis) after a simple aqueous workup. The azides [(S,S)-13] thus obtained were reacted in

(S,S)-7a, 35 %

(S,S)-7b, 38 %

(S,S)-7c, 29 %

(S,S)-7d, 87 %

(S,S)-7e, 50 %
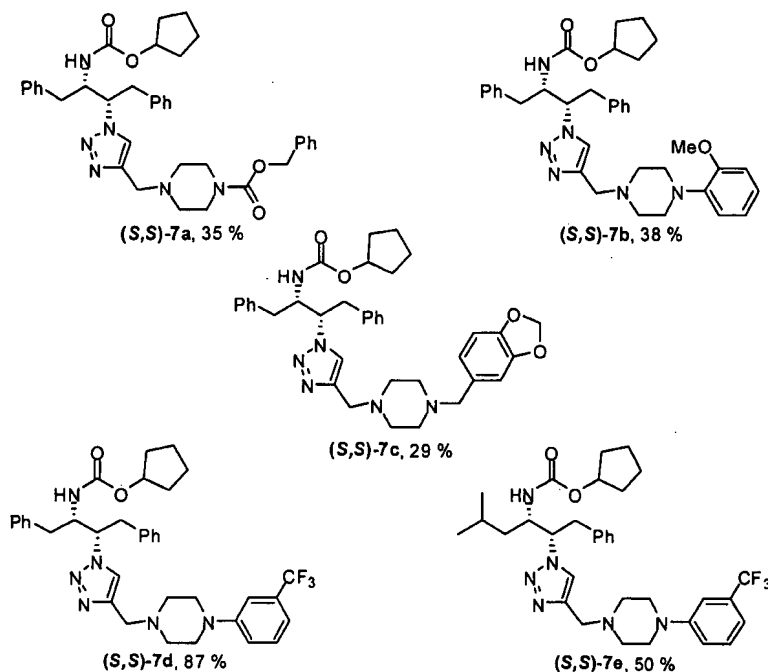
Figure 1. Structures and percentage inhibition of HIV-1-Pr for initial hit compounds at 5 μM concentration of the crude reaction mixtures.

Scheme 8. Synthesis and K_i Values of Triazole Products

(S,S)-6a, R = Bn
(S,S)-6c, R = ^{i}Bu

(S,S)-7d, R = Bn 54 % K_i = 98 ± 2 nM
(S,S)-7e, R = ^{i}Bu 63 % K_i = 86 ± 9 nM
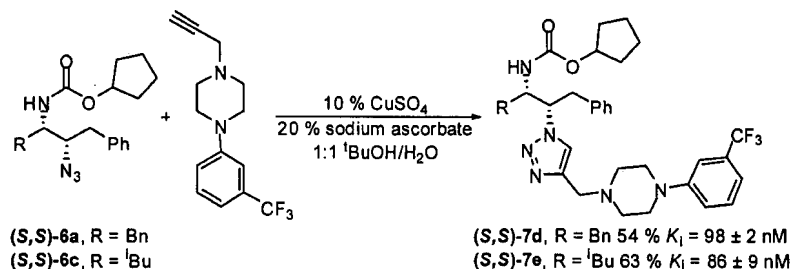
Scheme 9. Exploration of the Azide-Containing Fragment

(S,S)-12a

$R \overset{O}{\underset{}{\parallel}} Cl$, R–NCO, $R \cdot O \overset{O}{\underset{}{\parallel}} Cl$ or $R \cdot O \overset{O}{\underset{}{\parallel}} O$-N$\overset{}{\underset{}{}}$

triethanolamine, PhMe/H$_2$O

(S,S)-13

X = O, NH, CH, CH$_2$
R = Alk, c-Alk, Ar, het-Ar

14

CuSO$_4$, Cu$_{(s)}$
^{t}BuOH/H$_2$O 1:1

(S,S)-15
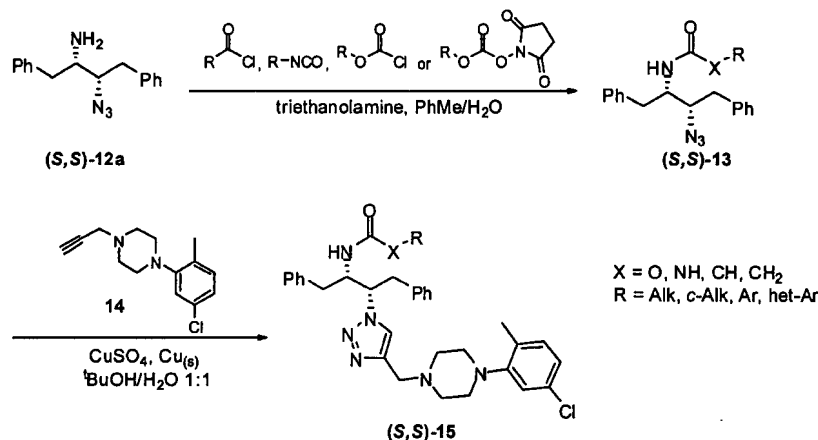
Table 1. Percentage Inhibition of HIV-1 PR by Selected Crude Reaction Mixtures at 5 μM and K_i Values for Selected Isolated Products

(S,S)-6a

CuSO$_4$, Cu$_{(s)}$
^{t}BuOH/H$_2$O 1:1
50 °C, 5 days

(S,S)-11

compd	R	% inhibition	compd	R	% inhibition	K_i
a		82	g		76	
b		67	h		93	
c		70	i		77	
d		22	j		94	23 ± 4 nM
e		20	k		94	32 ± 3 nM
f		12				

Table 2. Percentage Inhibition of Selected Crude Reaction Mixtures at 5 μM and K_i Values for Selected Isolated Products

(S,S)-15

compd	X-R	% inhibition	K_i
a		28	>2.5 μM
b		19	
c		73	
d		76	
e		71	83 ± 14 nM
f		31	

parallel with alkyne 14, as described above, to form a further array of triazoles with general structure (S,S)-15 (Scheme 9).

The crude reaction mixtures were again diluted to 5 μM and screened directly for HIV-1-PR inhibition (selected examples are shown in Table 2). Surprisingly, none of the amide or urea derivatives showed any observable inhibition at 5 μM [for example, Table 2, compounds (S,S)-15a,b]. A number of small alkyl carbamate derivatives [(S,S)-15c−e] showed similar activ-

Figure 2. Inhibitor (*S,S*)-**11j** computationally docked into the HIV-1-Pr active site, showing hydrogen bonds from the triazole *N*-3 and carbamate oxygen to the active-site water as green spheres. Protease flap regions are removed for clarity.
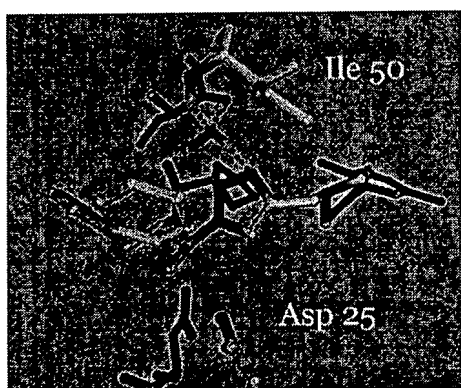
Figure 3. Detail of inhibitor (*S,S*)-**11j** computationally docked into the HIV-1-Pr active site. Protease amino acids Asp25, Asp25', Ile50, and Ile50' are shown. Hydrogen bonds between the ligand and protease, and the active-site water are shown as green spheres. The triazole is positioned in proximity to the catalytic aspartic acid residues, suggesting possible beneficial interactions upon incorporation of a small polar group at *C*-5.

ity to (*S,S*)-**11j** (94% inhibition at 5 μM), but incorporation of an aryl group led to much reduced activity [(*S,S*)-**15f**]. To confirm these results, several of the inhibitors were synthesized and isolated, and their K_i values were determined (Table 2). None of the capping groups we tried gave compounds that exhibited as high a protease inhibition as (*S,S*)-**11j**, which contained the initially chosen cyclopentyl carbamate.
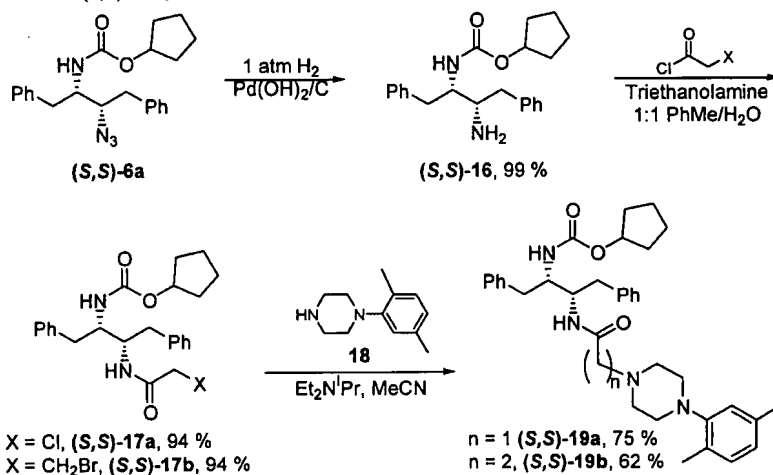
It was noteworthy that the azide and the alkyne fragments from which the most active compound [*S,S*)-**11j**] was assembled exhibited only minimal inhibitory activity themselves (azide (*S,S*)-**4a** $K_i = 31 \pm 22 \mu$M, alkyne **14** showed no observable inhibition even at 250 μM); the azide, which was the more active of the two building blocks, was over 1000 times less active than the final product. Computational docking of (*S,S*)-**11j** into the protease active site by use of AutoDock indicated that the compound adopted a conformation in which *N*-3 of the triazole and the carbonyl oxygen of the carbamate formed hydrogen bonds with the water molecule in the active site (Figure 2). Furthermore, no direct interaction between the inhibitor and the two aspartate residues in the active site was observed. This was in stark contrast to the currently licensed inhibitors, all of which contain a polar group, for example a hydroxyl, that acts as a transition-state mimic and strongly interacts with these residues. We reasoned that incorporation of a suitably positioned small polar group into our inhibitors could enable interaction with the aspartates, thereby increasing the binding energy (Figure 3). We envisioned two strategies for the rapid synthesis of analogues to accomplish this task: (a) replacement of the triazole with alternative, readily formed linkers and (b) direct modification of the 1,4-triazole unit to incorporate additional functionality.

Since the 1,2,3-triazole heterocycle has been shown to be a viable isostere of the amide bond,[18] we were interested to see whether replacement of the triazole in (*S,S*)-**11j** with a two- or three-carbon amide-containing linker would still give effective protease inhibition. We chose to base our initial analogues on compound (*S,S*)-**11k**, which contained a 2,5-dimethylphenylpiperazine, rather than the slightly more active chloro derivative (*S,S*)-**11j** to avoid possible complications with strongly basic reaction conditions we planned to use later. The initial catalytic hydrogenation of the azide (*S,S*)-**4a** with Pd(OH)$_2$/C gave the free amine (*S,S*)-**16** in 99% yield. Acylation with chloroacetyl chloride or 3-bromopropionyl chloride then furnished the halogenated amides (*S,S*)-**17a,b**, which were directly reacted with arylpiperazine **18** to furnish the desired compounds (*S,S*)-**19a,b** in high yield (Scheme 10). The isomeric amides (*S,S*)-**20a,b** were readily prepared in a similar fashion (Scheme 11).

The HIV-1-PR inhibition assay revealed that all four amide derivatives exhibited greatly reduced activity compared to the parent triazole: only minimal protease inhibition was observed at 25 μM concentration. We also synthesized the 1,5-disubstituted triazole regioisomer (*S,S*)-**21** in a selective manner, using the recently developed ruthenium-catalyzed triazole formation

Scheme 10. Synthesis of Amides (*S,S*)-**19a,b**

X = Cl, (*S,S*)-**17a**, 94 %
X = CH$_2$Br, (*S,S*)-**17b**, 94 %

n = 1 (*S,S*)-**19a**, 75 %
n = 2, (*S,S*)-**19b**, 62 %

Scheme 11. Synthesis of Amides (*S,S*)-20a,b

n = 1 (*S,S*)-20a, 31 %
n = 2, (*S,S*)-20b, 50 %
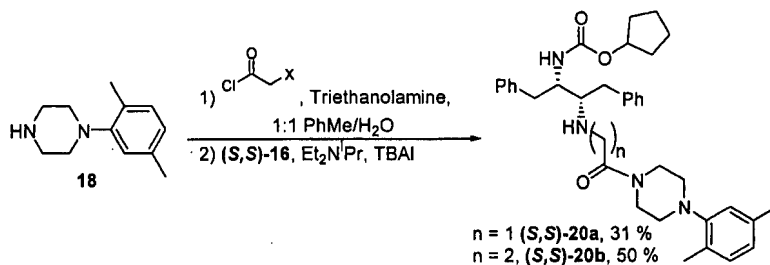
Scheme 12. Synthesis of 1,5-Disubtituted Triazole (*S,S*)-21
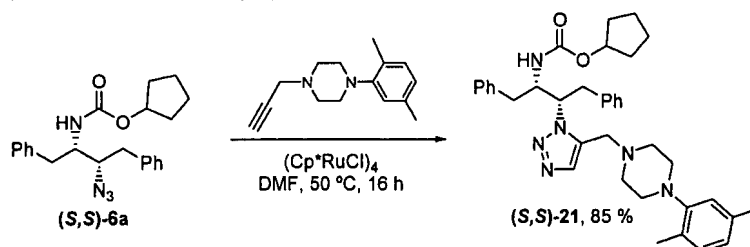
Table 3. Direct Functionalization of 1,4-Triazoles at the 5-Position

product	R^1	electrophile	R^2	yield (%)	K_i
(*S,S*)-22a	Me	TMS-Cl	TMS	58	>2.5 μM
(*S,S*)-22b	Me	*p*-CH$_2$O	-CH$_2$OH	53	14 ± 1 nM
(*S,S,S*)-22c	Me	MeCHO	(*S*)-CH(OH)Me	65[a]	10 ± 1 nM[b]
(*S,S,R*)-22c	Me	MeCHO	(*R*)-CH(OH)Me	23[c]	25 ± 3 nM[d]
(*S,S,S*)-22d	Me	EtCHO	(*S*)-CH(OH)Et	45[a]	27 ± 1 nM[b]
(*S,S,R*)-22d	Me	EtCHO	(*R*)-CH(OH)Et	15[c]	42 ± 5 nM[d]
(*S,S*)-24b	Cl	*p*-CH$_2$O	-CH$_2$OH	38	8 ± 0.5 nM
(*S,S,S*)-24c	Cl	MeCHO	(*S*)-CH(OH)Me	58[a]	21 ± 2 nM[b]
(*S,S,R*)-24c	Cl	MeCHO	(*R*)-CH(OH)Me	16[c]	28 ± 6 nM[d]

[a] Isolated yield of major, least polar *S,S,S* alcohol isomer. [b] K_i of major, least polar, *S,S,S* alcohol isomer. [c] Isolated yield of minor, most polar, *S,S,R* alcohol isomer. [d] K_i or minor, most polar, *S,S,R* alcohol isomer.

reaction (Scheme 12).[19] Unsurprisingly, this compound also exhibited much lower protease inhibition (IC$_{50}$ > 25 μM) than the parent 1,4-isomer.

These results indicated that the 1,4-disubstituted triazole linkage was crucial for efficient binding to the active-site residues. Therefore, we chose to install an additional small and polar substituent at the triazole C-5 position by direct quenching of a 5-metalated species. It has been previously demonstrated that a range of *N*-1-substituted triazoles can be selectively metalated at the 5-position and subsequently functionalized with suitable electrophiles.[20] There are also several isolated examples of the metalation of 1,4-disubstituted triazoles at the 5-position.[20a,21] However, these studies were carried out on very simple, minimally functionalized substrates. Furthermore, all of them contained at least one aryl substituent directly bonded to the triazole ring. The recent discovery of selective routes for the formation of 1,4-disubstituted triazoles[6] combined with direct deprotonation and quenching at C-5 creates a powerful method for the selective synthesis of 1,4,5-trisubstituted triazoles. We were therefore pleased to find that treatment of (*S,S*)-11k with 2.2 equiv of *n*-BuLi at −78 °C gave the dianion, which could be trapped with trimethylsilyl chloride (TMS-Cl) to give the 5-silylated triazole (*S,S*)-22a as the major product (Table 3). A small amount of disilylated product incorporating a second TMS group on the carbamate nitrogen was also isolated, but it could be readily converted to (*S,S*)-22a by stirring with aqueous HCl. No alternative products with silicon incorporated at any of the five potentially acidic benzylic positions were observed. Quenching of the dianion with paraformaldehyde gave the hydroxymethyl derivative (*S,S*)-22b. Two further hydroxyl-containing derivatives, 22c,d, were synthesized by quenching of the dianion
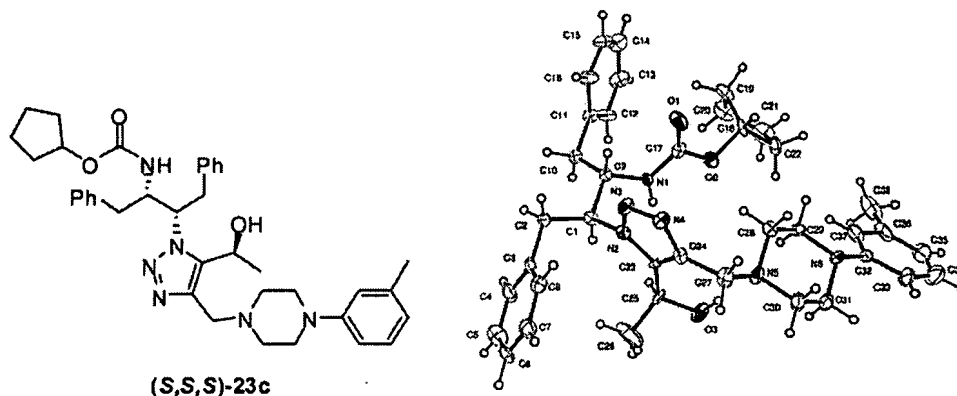
(*S,S,S*)-23c

Figure 4. Compound (*S,S,S*)-23c structure and ORTEP drawing derived from X-ray crystallographic analysis.

with acetaldehyde or propionaldehyde, respectively. These latter reactions gave rise to readily separable mixtures of the two possible diastereomeric alcohols favoring the all-*S* products (*S,S,S*)-**22c,d** over (*S,S,R*)-**22c,d** by approximately 3:1. The configurations of the diastereomeric products were assigned after acquisition of an X-ray crystal structure of a closely related analogue (*S,S,S*)-**23c** (Figure 4).[16] Selective deprotonation was even possible in the presence of the reactive aryl chloride in (*S,S*)-**11j**; the triazolyllithium reagent was then quenched with paraformaldehyde or acetaldehyde, yielding (*S,S*)-**24b** or (*S,S,S*)- and (*S,S,R*)-**24c**, respectively (Table 3). As predicted from the docking calculations, incorporation of small polar groups gave increased inhibitory activity: the 5-hydroxymethyl compounds, (*S,S*)-**22b** and (*S,S*)-**24b**, were approximately 3 times more potent than the corresponding 5-unsubstituted triazoles. The compounds containing the larger hydroxyethyl or hydroxypropyl substituents (**22c,d**, and **24c**) tended to give slightly weaker inhibition, with the major *S,S,S*, isomers being more active than the minor *S,S,R* diastereomers.

Conclusions

Among many factors that determine success of a combinatorial search for novel compounds with desired activity, two stand out: the degree of *diversity* of the blocks that can be brought to bear and the *speed* with which synthesis, screening for function, and lead optimization can be performed. The greater the variety of scaffolds and functional groups that can be employed in the *rapid* construction of candidate compounds, the more likely it is that new and useful function will be discovered. In this study, the fidelity and robustness of the CuAAC process enabled the discovery of a novel series of HIV-1 protease inhibitors with low nanomolar activities. It was used to assemble the initial screening library from diverse arrays of highly functionalized azide- and alkyne-containing fragments. Since no protecting groups were required and byproducts were minimal, the crude reaction mixtures were screened directly for protease inhibition, leading to rapid identification of fragments that produced highly potent inhibitors upon their unification. The optimization of the initial hits was accomplished by repetition of this process. Replacement or modification of the triazole fragment in the hit compounds revealed that it is more than a simple connector and is, in fact, essential for the activity of the hits. For example, its replacement with an amide greatly reduced the potency of the candidate compounds, whereas functionalization of the triazole ring itself led to inhibitors with increased potency. This general strategy of using the versatile CuAAC reaction to discover and optimize the initial fragments, followed by replacement or derivatization of the 1,4-triazole linkage to maximize the compound's desired properties, should be useful in facilitating rapid discovery of novel lead structures against a wide range of targets.

Experimental Section

General Procedures. Reagents and solvents were purchased from commercial sources and were used as received. Reaction progress was monitored by thin-layer chromatography (TLC) on Merck silica gel 60 F-254 with detection by UV. Silica gel 60 (Merck 40−63 μm) was used for column chromatography. Melting points are uncorrected and were determined by use of a Thomas-Hoover, uni-melt, capillary melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker DRX-600, Bruker DRX-500, or Bruker AMX-400 spectrometers. Proton magnetic resonance (^{1}H NMR) spectra were recorded at 600, 500, or 400 MHz. Data are presented as follows: chemical shift (parts per million, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q

= quartet, quin = quintet, sep = septet, m = multiplet, br = broad), coupling constant, *J* (hertz), and integration. Carbon magnetic resonance (^{13}C NMR) spectra were recorded at 150, 125, or 100 MHz. Data for ^{13}C NMR are reported in terms of chemical shifts (parts per million, ppm), and multiplicity (as above) followed by coupling constant (hertz) for fluorine-containing compounds. Most of the carbamate-containing products described here are observable by NMR at room temperature as mixtures of carbamate rotamers, generally in ratios of over 9:1. In these cases only signals for the major rotamer are listed. The signals for the pairs of rotameric compounds coalesced when the spectra were recorded at elevated temperatures; however, this often resulted in very broad signals. High-resolution mass spectra (HRMS) were recorded at the mass spectrometry facility at The Scripps Research Institute, La Jolla, CA. Elemental analyses were performed by Midwest Microlab, LLC, Indianapolis, IN. X-ray crystallographic analysis was carried out at the University of California, San Diego small molecule X-ray crystallography facility. Enantiomeric excesses were determined by chiral HPLC analysis. Assays were performed on a Shimadzu A10 system equipped with a diode array detector. Details of columns, eluent systems, and retention times are given in the respective experimental procedures. HPLC homogeneities were determined by use of an Agilent 1100 LC/MSD with an Agilent 1100 SL mass spectrometer. System A: Zorbax 4.6 mm × 30 mm, SB-C18 reverse-phase column, preceded by a Phenomenex C18 guard column, eluting with 10−100% MeCN (+0.05% TFA) in 0.05% TFA, linear gradient over 10 min and then isocratic for 5 min, 0.5 mL/min flow rate with UV detection at 254 nm. System B: Zorbax 4.6 mm × 150 mm, SB-C18 reverse-phase column, preceded by a Phenomenex C18 guard column, eluting with 10−100% MeOH (+0.05% TFA) in 0.05% TFA, linear gradient over 10 min and then isocratic for 10 min, 0.5 mL/min flow rate with UV detection at 254 nm. Fluorescence measurements were conducted on a Hitachi F2000 fluorescence spectrophotometer or a Packard fluorescence spectrophotometer (Fusion universal microplate analyzer) for microtiter plate assays.

Computational Docking Protocol. Atomic coordinates for the HIV-1 protease were obtained from the Protein Data Bank, PDB code 1HPV.[22] The ligand and crystallographic waters were removed with the exception of the water bridging the flaps. Polar hydrogens were added and Kollman charges were assigned to all atoms. Affinity grids centered on and encompassing the active site were calculated with 0.375 Å spacing by use of Autogrid3.[8] For the ligands, atomic coordinates were generated and initial molecular conformations minimized by use of ChemBats3D (CambridgeSoft). Gasteiger charges were assigned to all atoms and rotatable bonds were assigned with AutoDockTools. Autodock version 3.0.5 was used to evaluate ligand binding energies over the conformational search space by use of the Lamarckian genetic algorithm. Default docking parameters were used with the following exceptions: ga_pop_size, 150; ga_num_evals, 1750000; ga_run, 20.

[(1*S*,2*S*)-2-Azido-1,2-dibenzylethyl]carbamic Acid *tert*-Butyl Ester [(*S,S*)-4a].[13] To a solution of (*S*)-**3a** (2.71 g, 8.80 mmol, 1 equiv) in THF (24 mL) at 0 °C was added BnMgCl (20.2 mL of a 1.31 M solution in THF, 26.4 mmol, 3.0 equiv). The reaction mixture was slowly allowed to warm to room temperature and stirred for a further 3 h before being poured into 1 M HCl (15 mL). The mixture was extracted with Et₂O (3 × 20 mL), and the combined organic extracts were dried over MgSO₄ before being concentrated under reduced pressure to furnish the crude product. Flash chromatography (20% EtOAc in hexanes) furnished the desired ketone[12] (2.86 g, 96%).

To a solution of the ketone obtained above (2.81 g, 8.29 mmol, 1 equiv) in MeOH (58 mL) at −22 °C was added NaBH₄ (0.94 g, 25 mmol, 3 equiv). The reaction was maintained at −22 °C for 30 min and then allowed to warm to room temperature before being quenched by pouring into a saturated solution of NH₄Cl (60 mL). The reaction mixture was extracted with Et₂O (4 × 100 mL), and the combined organic extracts were washed with 1 M HCl (100 mL) and saturated NaHCO₃ solution (100 mL) before being concentrated under vacuum to furnish the crude amino alcohol **2a**

as a 9:1 mixture of diastereomers in favor of the anti isomer[12] as determined by ^{1}H NMR. Recrystallization from benzene afforded the pure anti isomer (*S,R*)-**2a** (1.82 g, 64%).

A solution of the amino alcohol (*S,R*)-**2a** obtained above (534 mg, 1.57 mmol, 1.0 equiv) in DCM (16 mL) was cooled to 0 °C before being treated with Et₃N (1.16 mL, 8.34 mmol, 1.2 equiv) followed by MsCl (592 µL, 17.7 mmol, 1.1 equiv). The reaction was maintained at 0 °C for 1 h and then allowed to warm to room temperature and stirred for a further 1 h before being diluted with DCM (35 mL) and washed with 1 M HCl (50 mL), saturated NaHCO₃ solution (50 mL), and finally brine (50 mL). The organic portion was dried over MgSO₄ and concentrated under reduced pressure before being purified by flushing through a short pad of silica (20% EtOAc in hexanes) to furnish the desired mesylate (597 mg, 91%).

To a solution of the mesylate obtained above (1.65 g, 3.93 mmol, 1.0 equiv) in DMF (20 mL) was added NaN₃ (358 mg, 5.50 mmol, 1.4 equiv). The reaction was heated to 60 °C for 16 h before being cooled to room temperature, diluted with Et₂O (60 mL), and washed with H₂O (5 × 60 mL) followed by brine (60 mL). The organic portion was dried over MgSO₄ and concentrated under reduced pressure before being purified by flash chromatography (10% EtOAc in hexanes) to furnish the title compound as a white solid (956 mg, 64%; mp 49−50 °C, ee > 98%[23]). [α]_D +3.2 (*c* 1.0, CDCl₃); ^{1}H NMR (600 MHz, CDCl₃) δ = 7.30−7.14 (m, 10H), 4.72 (d, *J* = 9.6, 1H), 4.00 (br q, *J* = 7.8, 1H), 3.59 (br t, *J* = 6.6, 1H), 2.92 (dd, *J* = 13.8 and 6.6, 1H), 2.86 (d, *J* = 7.2, 2H), 2.77 (dd, *J* = 13.8 and 9.0, 1H), 1.43 (s, 9H); ^{13}C NMR (150 MHz, CDCl₃) δ = 155.4, 137.3, 137.1, 129.3, 129.1, 128.6, 128.6, 126.9, 126.6, 79.6, 65.5, 53.7, 39.8, 38.5, 28.3; HRMS [electrospray ionization (ESI)−time of flight (TOF)] calculated for C₂₁H₂₆N₄NaO₂ (MNa⁺) 389.1948, found 389.1950.

[(1*R*,2*R*)-2-Azido-1,2-dibenzylethyl]carbamic Acid *tert*-Butyl Ester [(*R,R*)-4a]. The procedure for the synthesis of (*S,S*)-**4a** was followed, starting from (*R*)-**3a** in place of its enantiomer. Thus the ketone was obtained in 86% yield and subsequently reduced with NaBH₄ to give, after recrystallization from benzene, the pure anti amino alcohol (*R,S*)-**2a** in 64% yield. Reaction with MsCl furnished the mesylate in 82% yield. Reaction with NaN₃ then gave the title compound as a white solid (66%; mp 50−51 °C, ee > 98%[23]). [α]_D −3.1 (*c* 1.0, CDCl₃); HRMS (ESI-TOF) calculated for C₂₁H₂₆N₄NaO₂ (MNa⁺) 389.1948, found 389.1947. All other data were identical to those for (*S,S*)-**4a**.

[(1*S*,2*S*)-2-Azido-1-benzyl-2-isobutylethyl]carbamic Acid *tert*-Butyl Ester [(*S,S*)-4b]. The procedure for the synthesis of (*S,S*)-**4a** was followed with [i]BuMgCl used in place of BnMgCl. Thus the ketone was obtained after flash chromatographic purification (15% EtOAc in hexanes) in 90% yield and subsequently reduced with NaBH₄ to give the amino alcohol **2b** as a 5:1 mixture of diastereomers in favor of the anti isomer (*S,R*)-**2b**. Recrystallization from benzene furnished the pure anti isomer (*S,R*)-**2b** in 57% yield. Reaction with MsCl furnished the mesylate in 65% yield. Reaction with NaN₃ then gave the title compound as a white solid (58%; mp 74−75 °C). [α]_D +4.8 (*c* 1.0, CDCl₃); ^{1}H NMR (600 MHz, CDCl₃) δ = 7.32−7.22 (m, 5H), 4.63 (d, *J* = 9.6, 1H), 3.93 (br q, *J* = 7.8, 1H), 3.44 (br t, *J* = 7.2, 1H), 2.91 (dd, *J* = 13.8 and 6.6, 1H), 2.77 (dd, *J* = 13.8 and 9.0, 1H), 1.72 (sep, *J* = 6.6, 1H), 1.47−1.40 (m, 2H), 1.40 (s, 9H), 0.85 (d, *J* = 6.6, 3H), 0.77 (d, *J* = 6.6, 3H); ^{13}C NMR (150 MHz, CDCl₃) δ = 155.5, 137.6, 129.2, 128.6, 126.6, 79.5, 61.8, 53.9, 40.3, 39.6, 28.3, 24.8, 22.5, 22.2; HRMS (ESI-TOF) calculated for C₁₈H₂₈N₄NaO₂ (MNa⁺) 355.2104, found 355.2103.

[(1*S*,2*S*)-2-Azido-2-benzyl-1-isobutylethyl]carbamic Acid *tert*-Butyl Ester [(*S,S*)-4c]. The procedure for the synthesis of (*S,S*)-**4a** was followed, starting from (*S*)-**3c** in place of (*S*)-**3a**. Thus the ketone was obtained after flash chromatographic purification (10% EtOAc in hexanes) in 86% yield and subsequently reduced with NaBH₄ to give the amino alcohol **2c** as a 7:1 mixture of diastereomers in favor of the anti isomer (*S,R*)-**2c**. Recrystallization from hexanes furnished the pure anti isomer (*S,R*)-**2c** in 74% yield. Reaction with MsCl furnished the mesylate in 92% yield. Reaction

with NaN₃ then gave, after flash chromatographic purification (5% EtOAc in hexanes), the title compound as a white solid (64%; mp 51−53 °C). [α]_D −42.9 (*c* 1.0, CDCl₃); ^{1}H NMR (600 MHz, CDCl₃) δ = 7.34−7.24 (m, 5H), 4.53 (d, *J* = 9.6, 1H), 3.87−3.83 (m, 1H), 3.61 (ddd, *J* = 8.4, 6.0, and 2.4, 1H), 2.91 (dd, *J* = 13.8 and 6.0, 1H), 2.81 (dd, *J* = 13.8 and 9.0, 1H), 1.65−1.58 (m, 1H), 1.49−1.44 (m, 1H), 1.47 (s, 9H), 1.29 (ddd, *J* = 13.8, 8.4, and 5.4, 1H), 0.91 (d, *J* = 6.6, 3H), 0.77 (d, *J* = 7.2, 3H); ^{13}C NMR (150 MHz, CDCl₃): δ = 155.6, 137.6, 129.4, 128.6, 126.8, 79.4, 68.2, 50.9, 42.6, 38.4, 28.4, 24.8, 22.9, 22.2; HRMS (ESI-TOF) calculated for C₁₈H₂₈N₄NaO₂ (MNa⁺) 355.2104, found 355.2107.

[(1*S*,2*S*)-2-Azido-1,2-diisobutylethyl]carbamic Acid *tert*-Butyl Ester [(*S,S*)-4d]. The procedure for the synthesis of (*S,S*)-**4c** was followed, with [i]BuMgCl in place of BnMgCl. Thus the ketone was obtained after flash chromatographic purification (10% EtOAc in hexanes) in 65% yield and subsequently reduced with NaBH₄ to give the amino alcohol as a single anti diastereomer. Recrystallization from benzene furnished the pure anti compound (*S,R*)-**2c** in 67% yield. Reaction with MsCl furnished the mesylate in 91% yield. Reaction with NaN₃ then gave, after flash chromatographic purification (5% EtOAc in hexanes), the title compound as a white solid (45%; mp 41−42 °C). [α]_D −39.5 (*c* 1.0, CDCl₃); ^{1}H NMR (600 MHz, CDCl₃) δ = 4.42 (d, *J* = 9.6, 1H), 3.79−3.75 (m, 1H), 3.61 (m, 1H), 1.81 (sep, *J* = 6.6, 1H), 1.65 (sep, *J* = 6.6, 1H), 1.49−1.26 (m, 4H), 1.43 (s, 9H), 0.94−0.92 (m, 12H); ^{13}C NMR (150 MHz, CDCl₃) δ = 155.7, 79.3, 64.6, 51.2, 42.5, 40.3, 28.3, 24.9, 23.1, 22.8, 22.3, 22.2; HRMS (ESI-TOF) calculated for C₁₅H₃₀N₄NaO₂ (MNa⁺) 321.2261, found 321.2260.

[(1*S*,2*R*)-2-Azido-1,2-dibenzylethyl]carbamic Acid *tert*-Butyl Ester [(*S,R*)-4a]. To a suspension of CuBr·DMS (6.60 g, 32.1 mmol, 2.0 equiv) in THF (80 mL) at −78 °C was added dropwise BnMgCl (49.1 mL of a 1.31 M solution in THF, 64.2 mmol, 4.0 equiv). After complete addition, the resulting solution was stirred for a further 10 min at −78 °C before a solution of (*S*)-**5a**[24] (4.00 g, 16.1 mmol, 1.0 equiv) in THF (60 mL + 20 mL wash) was added dropwise by cannula. The reaction was maintained at −78 °C for 10 min and then allowed to warm to room temperature and stirred for a further 3h before being quenched by the addition of saturated ammonium chloride solution (250 mL). The flask was opened to the atmosphere and vigorously stirred until a deep blue coloration had developed (~1 h). The resulting biphasic suspension was filtered through a short pad of Celite to remove any precipitated solids before being extracted with Et₂O (3 × 200 mL). The combined organic extracts were dried over MgSO₄ before being concentrated under reduced pressure to furnish the crude alcohol **2a** as a 5:1 mixture of diastereoisomers in favor of the desired syn isomer (*S,S*)-**2a** as determined by ^{1}H NMR. Flash chromatography (25% EtOAc in hexanes) enabled isolation of the pure syn isomer (*S,S*)-**2a** (2.59 g, 47%).

The product obtained above (2.37 g, 6.95 mmol, 1.0 equiv) was dissolved in DCM (35 mL) and cooled to 0 °C before being treated with Et₃N (1.16 mL, 8.34 mmol, 1.2 equiv) followed by MsCl (592 µL, 7.65 mmol, 1.1 equiv). The reaction was maintained at 0 °C for 1 h and then allowed to warm to room temperature and stirred for a further 1 h before being diluted with DCM (35 mL) and washed with 1 M HCl (50 mL), saturated NaHCO₃ solution (50 mL) and finally brine (50 mL). The organic portion was dried over MgSO₄ and concentrated under reduced pressure before being purified by flushing through a short pad of silica (20% EtOAc in hexanes) to furnish the desired mesylate (2.50 g, 86%).

To a solution of the mesylate obtained above (2.41 g, 5.75 mmol, 1.0 equiv) in DMF (30 mL) was added NaN₃ (468 mg, 7.99 mmol, 1.4 equiv). The reaction was heated to 60 °C for 16 h before being cooled to room temperature, diluted with Et₂O (100 mL), and washed with H₂O (5 × 100 mL) followed by brine (100 mL). The organic portion was dried over MgSO₄ and concentrated under reduced pressure before being purified by flash chromatography (12.5% EtOAc in hexanes) to furnish the title compound as a white solid (1.64 g, 78%; mp 127−128 °C, ee > 98%[25]). [α]_D −38.0 (*c* 1.0, CDCl₃); ^{1}H NMR (600 MHz, CDCl₃) δ = 7.35−7.17 (m, 10H), 4.57 (br d, *J* = 7.2, 1H), 3.99 (br s, 1H), 3.38 (br s, 1H), 3.02 (dd,

J = 14.4 and 3.6, 1H), 2.98 (dd, J = 14.4 and 4.2, 1H), 2.85−2.72 (m, 2H), 1.34 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ = 155.1, 137.3, 137.3, 129.2, 129.2, 128.7, 128.5, 127.0, 126.6, 79.7, 67.1, 54.5, 38.0, 35.8, 28.2; HRMS (ESI-TOF) calculated for C$_{21}$H$_{26}$N$_4$-NaO$_2$ (MNa$^+$) 389.1948, found 389.1950.

[(1*R*,2*S*)-2-Azido-1,2-dibenzylethyl]carbamic Acid *tert*-Butyl Ester [(*R*,*S*)-4a]. The procedure for the synthesis of (*S*,*R*)-4a was followed, starting from (*S*)-5a^{24} in place of its enantiomer. Thus, the syn amino alcohol (*R*,*R*)-2a was obtained in 60% yield. Reaction with MsCl furnished the mesylate in 61% yield. Reaction with NaN$_3$ then gave the title compound (47%; mp 126−127 °C, ee > 98%^{25}). [α]$_D$ +37.4 (*c* 1.0, CDCl$_3$); HRMS (ESI-TOF) calculated for C$_{21}$H$_{26}$N$_4$NaO$_2$ (MNa$^+$) 389.1948, found 389.1952. All other data were identical to those for (*S*,*R*)-4a.

[(1*S*,2*R*)-2-Azido-1-benzyl-2-isobutylethyl]carbamic Acid *tert*-Butyl Ester [(*S*,*R*)-4b]. To a solution of (*S*)-5a^{24} (4.13 g, 1.66 mmol, 1.0 equiv) in THF (166 mL) at room temperature was added dropwise ^{i}BuMgCl (24.9 mL of a 2.0 M solution in THF, 49.8 mmol, 3.0 equiv). After complete addition, the resulting solution was stirred for a further 2.5 h before being quenched by the addition of saturated ammonium chloride solution (100 mL). The reaction mixture was extracted with DCM (3 × 200 mL) and the combined organic portions were washed with brine (200 mL) before being dried over MgSO$_4$ and concentrated under reduced pressure to furnish the crude alcohol 2b as a 7:1 mixture of diastereoisomers in favor of the desired syn isomer (*S*,*S*)-2b as determined by ^{1}H NMR. Flash chromatography (17.5% EtOAc in hexanes) enabled isolation of the pure syn isomer (*S*,*S*)-2b (2.37 g, 47%).

A solution of the amino alcohol (*S*,*S*)-2b obtained above (1.97 g, 6.25 mmol, 1.0 equiv) in DCM (31 mL) was cooled to 0 °C before being treated with Et$_3$N (1.04 mL, 7.50 mmol, 1.2 equiv) followed by MsCl (533 μL, 6.88 mmol, 1.1 equiv). The reaction was maintained at 0 °C for 1 h and then allowed to warm to room temperature and stirred for a further 1 h before being diluted with DCM (35 mL) and washed with 1 M HCl (50 mL), saturated NaHCO$_3$ (50 mL), and finally brine (50 mL). The organic portion was dried over MgSO$_4$ and concentrated under reduced pressure before being purified by flushing through a short pad of silica (20% EtOAc in hexanes) to furnish the desired mesylate (2.10 g, 87%).

To a solution of the mesylate obtained above (2.10 g, 5.44 mmol, 1.0 equiv) in DMF (30 mL) was added NaN$_3$ (495 mg, 7.60 mmol, 1.4 equiv). The reaction was heated to 60 °C for 16 h before being cooled to room temperature, diluted with Et$_2$O (100 mL), and washed with H$_2$O (5 × 100 mL) followed by brine (100 mL). The organic portion was dried over MgSO$_4$ and concentrated under reduced pressure before being purified by flash chromatography (12.5% EtOAc in hexanes) to furnish the title compound as a white solid (0.86 g, 48%; mp 93−94 °C, ee > 98%^{26}). [α]$_D$ −30.5 (*c* 1.0, CDCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ = 7.30−7.17 (m, 5H), 4.54 (br d, J = 7.2, 1H), 3.97 (br s, 1H), 3.62 (br s, 1H), 2.89 (dd, J = 14.4 and 4.8, 1H), 2.65 (dd, J = 13.8 and 10.2, 1H), 1.84−1.77 (m, 1H), 1.56−1.37 (m, 2H), 1.34 (s, 9H), 1.00 (d, J = 6.6, 3H), 0.94 (d, J = 6.6, 3H); ^{13}C NMR (150 MHz, CDCl$_3$): δ = 155.1, 137.5, 129.1, 128.5, 126.5, 79.6, 63.7, 54.8, 39.8, 35.7, 28.2, 25.3, 23.1, 22.0; HRMS (ESI-TOF) calculated for C$_{18}$H$_{28}$N$_4$NaO$_2$ (MNa$^+$) 355.2104, found 355.2100.

[(1*R*,2*S*)- 2-Azido-1-benzyl-2-isobutylethyl]carbamic Acid *tert*-Butyl Ester [(*R*,*S*)-4b]. The procedure for the synthesis of (*S*,*R*)-4b was followed, starting from (*R*)-5a^{24} in place of its enantiomer. Thus, the syn amino alcohol (*R*,*R*)-2b was obtained in 45% yield. Reaction with MsCl furnished the mesylate in 60% yield. Reaction with NaN$_3$ then gave the title compound (60%; mp 92−94 °C, ee > 98%^{26}). [α]$_D$ +32.6 (*c* 1.0, CDCl$_3$); HRMS (ESI-TOF) calculated for C$_{18}$H$_{28}$N$_4$NaO$_2$ (MNa$^+$) 355.2104, found 355.2103. All other data were identical to those for (*S*,*R*)-4b.

[(1*S*,2*R*)-2-Azido-2-benzyl-1-isobutylethyl]carbamic Acid *tert*-Butyl Ester [(*S*,*R*)-4c]. The procedure for the synthesis of (*S*,*R*)-4a was followed, starting from (*S*)-5c in place of (*S*)-5a. Thus, the crude amino alcohol 2c was obtained as a 5:1 mixture of diastereomers in favor of the syn isomer (*S*,*S*)-2c. Flash chromatographic separation (17.5% EtOAc in hexanes) gave the pure syn

isomer (*S*,*S*)-2c in 55% yield. Reaction with MsCl furnished the mesylate in 93% yield. Reaction with NaN$_3$ then gave, after flash chromatography (10% EtOAc in hexanes), the title compound as a white solid (76%; mp 72−73 °C). [α]$_D$ −47.3 (*c* 1.0, CDCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ = 7.34−7.24 (m, 5H), 4.54 (br d, J = 8.4, 1H), 3.80−3.75 (m, 2H), 2.85 (dd, J = 14.4 and 4.2, 1H), 2.77 (dd, J = 13.8 and 10.2, 1H), 1.71−1.65 (m, 1H), 1.47−1.31 (m, 2H), 1.45 (s, 9H), 0.96 (d, J = 6.6, 3H), 0.89 (d, J = 6.6, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ = 155.3, 137.5, 129.1, 128.7, 126.9, 79.6, 68.3, 52.1, 38.2, 37.9, 28.4, 24.7, 23.8, 21.4; HRMS (ESI-TOF) calculated for C$_{18}$H$_{28}$N$_4$NaO$_2$ (MNa$^+$) 355.2104, found 355.2107.

[(1*S*,2*R*)-2-Azido-1,2-diisobutylethyl]carbamic Acid *tert*-Butyl Ester [(*S*,*R*)-4d]. The procedure for the synthesis of (*S*,*R*)-4b was followed, starting from (*S*)-5c in place of (*S*)-5a. Thus, the crude amino alcohol 2d was obtained as an undetermined mixture of diastereomers. Flash chromatographic separation (13.5% EtOAc in hexanes) gave the pure syn isomer (*S*,*S*)-2d in 63% yield. Reaction with MsCl furnished the mesylate in 73% yield. Reaction with NaN$_3$ then gave, after flash chromatography (5% EtOAc in hexanes), the title compound as a white solid (68%; mp 38−39 °C). [α]$_D$ −48.6 (*c* 1.0, CDCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ = 4.52 (br d, J = 8.4, 1H), 3.75−3.70 (m, 1H), 3.56 (dt, J = 9.0 and 4.2, 1H), 1.79−1.72 (m, 1H), 1.68−1.62 (m, 1H), 1.49−1.43 (m, 1H),1.45 (s, 9H), 1.32−1.16 (m, 3H), 0.98 (d, J = 6.6, 3H), 0.95 (d, J = 6.6, 3H) 0.93 (d, J = 6.6, 3H), 0.90 (d, J = 6.6, 3H); ^{13}C NMR (150 MHz, CDCl$_3$): δ = 155.3, 79.5, 64.8, 52.2, 39.7, 38.1, 28.3, 25.3, 24.6, 23.8, 23.0, 22.1, 21.5; HRMS (ESI-TOF) calculated for C$_{15}$H$_{30}$N$_4$-NaO$_2$ (MNa$^+$) 321.2261, found 321.2259.

General Procedure for Synthesis of Carbamates 6 and Amides, Ureas, and Carbamates 13. To a solution of the *N*-Boc-α-amino azide 4 (1.0 equiv) in DCM (5 mL/mmol) at 0 °C was added TFA (5 mL/mmol). The reaction was stirred at 0 °C for 30 min before being quenched by cautiously pouring into a saturated solution of NaHCO$_3$. The biphasic mixture was extracted with DCM (2 × 30 mL/mmol) and the combined organic portions were dried over MgSO$_4$ before being concentrated under reduced pressure to give the free amino azide 12.

To a solution of the free amino azide 12 (1.0 equiv) obtained above, in toluene (10 mL/mmol), was added water (10 mL/mmol), followed by triethanolamine (1.2 equiv), and finally the required chloroformate, acyl chloride, isocyanate, or *O*-succinimidyl carbonate (1.1 equiv).^{16} The reaction was stirred at room temperature for 1 h before being diluted with EtOAc (30 mL/mmol) and washed sequentially with 1 M HCl (30 mL/mmol), NaHCO$_3$ (30 mL/mmol), water (30 mL/mmol), and brine (30 mL/mmol). The organic portion was then dried over MgSO$_4$ before being concentrated under reduced pressure to give the desired products 6 or 13 in 68−93% yield over the two steps, with >95% purity as judged by LC-MS analysis.

Library Synthesis and Analysis. A solution of azide (6) (100 μL of a 20 mM solution in ^{t}BuOH, 2 μmol, 1.0 equiv) was added to a glass reaction tube containing a solution of an alkyne^{16} (100 μL of a 24 mM solution in ^{t}BuOH, 2.4 μmol, 1.2 equiv). A solution of CuSO$_4$ (200 μL of a 0.5 mM solution in water, 0.1 μmol, 0.05 equiv) was added, followed by a small piece of copper turning to give final concentrations of: azide 5 mM, alkyne 6 mM, and CuSO$_4$ 0.25 mM in 1:1 ^{t}BuOH/H$_2$O. The reaction tubes were then sealed before being heated to 50 °C and shaken for 5 days. The crude reactions were analyzed by LC-MS, looking for disappearance of the azide starting material and formation of a product with the correct molecular weight. The crude reaction mixtures were then diluted to 0.5 mM with dimethyl sulfoxide (DMSO) (3.6 mL) and shaken gently until any precipitated products were redissolved. The remaining copper turning was removed and the crude DMSO solutions were further diluted to 5 μM into 96-well plates with 0.1 M MES and 0.2 M NaCl, pH 5.25 buffer, containing 5% (v/v) glycerol. To each well was added enzyme (20 μg/mL) and fluorogenic substrate (50 μM), before the plates were assayed directly for HIV-1-Pr inhibition. TL-3^{27} (100 nM) was used as a positive control.

Kinetic Determinations. IC_{50} values of the isolated inhibitors against HIV-1 protease SF-2-WTQ7K-Pr[27b,28] activity were assayed by use of the fluorogenic substrate Abz-Thr-Ile-Nle/Phe-(p-NO$_2$)-Gln-Arg-NH$_2$.[17] The initial rate of substrate hydrolysis was determined at different inhibitor concentrations (1 nM–25 μM; concentrations above 25 μM were generally not assayed due to insolubility problems with some fragments) with 50 μM substrate and 20 μg/mL enzyme at 37 °C, in 0.1 M MES and 0.2 M NaCl, pH 5.25 buffer, containing 5% (v/v) glycerol. IC_{50} values were determined from a dose–response plot of the obtained data by use of the GRAFIT program (version 3.0 Erithacus Software, U.K.). K_m and V_{max} values for the fluorogenic peptide described above were determined by measuring the initial rate of hydrolysis at different substrate concentrations (2.5–100 μM) and fitting the obtained data to the Michaelis–Menten equation by use of the GRAFIT program. K_i values for the isolated inhibitors were derived from the IC_{50} values via the formula for competitive inhibitor, $K_i = IC_{50}/(1 + [S]/K_m)$.

Cyclopentyl (2S,3S)-1,4-Diphenyl-3-(4-[(4-[3-(trifluoromethyl)phenyl]piperazin-1-yl)methyl]-1H-1,2,3-triazol-1-yl)butan-2-ylcarbamate [(S,S)-7d]. A solution of azide (S,S)-6a (50 mg, 0.13 mmol, 1.0 equiv) and 1-(prop-2-ynyl)-4-[3-(trifluoromethyl)phenyl]-piperazine (36 mg, 0.13 mmol, 1.0 equiv) in 'BuOH (1.3 mL) was treated with CuSO$_4$ (0.65 mL of a 0.02 M solution in H$_2$O, 0.013 mmol, 0.1 equiv) followed by sodium ascorbate (0.65 mL of a 0.04 M solution in H$_2$O, 0.026 mmol, 0.2 equiv). The reaction was stirred at room temperature for 16 h before being diluted with H$_2$O (15 mL); then 5 drops of 30% NH$_4$OH was added and the mixture was shaken vigorously for several minutes before being extracted with DCM (2 × 15 mL). The combined organic portions were washed with brine (15 mL) before being dried over MgSO$_4$ and concentrated under vacuum to give the crude product. Purification by flash chromatography (80–100% EtOAc in hexanes) furnished the title compound as a pale solid (53 mg, 63%; mp 148–150 °C). ^{1}H NMR (600 MHz, CDCl$_3$) δ = 7.28–6.79 (m, 15H), 6.10 (d, J = 9.6, 1H), 5.11–5.09 (m, 1H), 4.58–4.52 (m, 1H), 4.40–4.38 (m, 1H), 3.71 (br s, 2H), 3.34 (dd, J = 13.8 and 11.4, 1H), 3.19 (br s, 4H), 4.15 (dd, J = 13.8 and 3.6, 2H), 2.83 (dd, J = 13.8 and 6.6, 1H), 2.52 (br s, 4H), 2.38 (dd, J = 13.8 and 9.6, 1H), 1.87–1.57 (m, 8H); ^{13}C NMR (150 MHz, CDCl$_3$) δ = 156.5, 151.2 136.9, 136.7, 131.4 (q, J = 32), 129.5, 129.0, 128.8, 128.7, 128.6, 126.9, 126.8, 125.4, 124.2, (q, J = 272), 118.7, 115.9, 112.1, 103.0, 77.8, 64.5, 55.0, 52.9, 52.2, 48.5, 39.6, 39.3, 32.8, 32.7, 23.7, 23.6; HRMS (ESI-TOF) calculated for C$_{36}$H$_{42}$F$_3$N$_6$O$_2$ (MH$^+$) 647.3321, found 647.3321. Anal. (C$_{36}$H$_{41}$F$_3$N$_6$O$_2$) C, H, N.

Cyclopentyl (2S,3S)-3-[4-([4-(5-Chloro-2-methylphenyl)piperazin-1-yl]methyl)-1H-1,2,3-triazol-1-yl]-1,4-diphenylbutan-2-ylcarbamate [(S,S)-11j]. The procedure for the synthesis of (S,S)-7d was followed using 14 in place of 1-(prop-2-ynyl)-4-(3-(trifluoromethyl)phenyl)piperazine. Purification by flash chromatography on silica gel (50% EtOAc in hexanes) furnished the title compound as a pale pink solid (81%; mp 139–140 °C). ^{1}H NMR (500 MHz, CDCl$_3$) δ = 7.30–6.79 (m, 14H), 6.12 (d, J = 10.0, 1H), 5.11–5.08 (m, 1H), 4.58–4.52 (m, 1H), 4.39–4.37 (m, 1H), 3.72 (br s, 2H), 3.34 (dd, J = 14.0 and 11.0, 1H), 3.14 (dd, J = 14.0 and 4.0, 1H), 2.86 (br s, 4H), 2.83 (dd, J = 14.0 and 6.5, 1H), 2.52 (br s, 4H), 2.38 (dd, J = 13.5 and 9.0, 1H), 2.23 (s, 3H), 1.87–1.55 (m, 8H); ^{13}C NMR (125 MHz, CDCl$_3$) δ = 156.4, 152.4, 142.9, 136.9, 136.7, 131.9, 131.6, 130.7, 129.0, 128.8, 128.7, 128.5, 126.8, 126.8, 125.4, 122.9, 119.4, 77.8, 64.4, 55.0, 53.0, 52.8, 51.3, 39.6, 39.2, 32.8, 32.7, 23.6, 23.6, 17.4; HRMS (ESI-TOF) calculated for C$_{36}$H$_{44}$ClN$_6$O$_2$ (MH$^+$) 627.3214, found 627.3215. Anal. (C$_{36}$H$_{44}$ClN$_6$O$_2$) C, H, N.

Cyclopentyl (2S,3S)-3-[4-([4-(2,5-Dimethylphenyl)piperazin-1-yl]methyl)-1H-1,2,3-triazol-1-yl]-1,4-diphenylbutan-2-ylcarbamate [(S,S)-11k]. A solution of azide (S,S)-6a (200 mg, 0.53 mmol, 1.0 equiv) and 1-(2,5-dimethylphenyl)-4-(prop-2-ynyl)-piperazine (121 mg, 0.53 mmol, 1.0 equiv) in 'BuOH (5.3 mL) was treated with CuSO$_4$ (2.65 mL of a 0.02 M solution in H$_2$O, 0.053 mmol, 0.1 equiv) followed by sodium ascorbate (2.65 mL

of a 0.04 M solution in H$_2$O, 0.11 mmol, 0.2 equiv). The reaction was stirred for 24 h before being diluted with H$_2$O (30 mL) and NH$_4$OH (2 mL of a 30% aqueous solution), causing the product to precipitate. The mixture was stirred vigorously for 10 min before the precipitate was collected by filtration, washed with NH$_4$OH (30 mL of a 2 M aqueous solution) and water (30 mL). The collected product was dried under high vacuum to furnish the title compound without need for further purification, as a pale solid (313 mg, 98%; mp 148–150 °C). ^{1}H NMR (500 MHz, CDCl$_3$) δ = 7.30–6.79 (m, 14H), 6.13 (d, J = 10.0, 1H), 5.12–5.08 (m, 1H), 4.59–4.53 (m, 1H), 4.40–4.36 (m, 1H), 3.71 (s, 2H), 3.35 (dd, J = 14.0 and 11.0, 1H), 3.14 (dd, J = 14.0 and 4.0, 1H), 2.89 (br s, 4H), 2.84 (dd, J = 14.0 and 6.5, 1H), 2.52 (br s, 4H), 2.38 (dd, J = 14.0 and 9.0, 1H), 2.31 (s, 3H), 2.25 (s, 3H), 1.92–1.55 (m, 8H); ^{13}C NMR (125 MHz, CDCl$_3$) δ = 156.5, 151.2, 143.1, 137.0, 136.7, 136.0, 130.8, 129.3, 129.0, 128.8, 128.7, 128.5, 126.8, 126.8, 125.3, 123.8, 119.7, 77.8, 64.4, 55.0, 53.1, 53.1, 51.5, 39.6, 39.3, 32.8, 32.7, 23.7, 23.6, 21.2, 17.4; HRMS (ESI-TOF) calculated for C$_{37}$H$_{47}$N$_6$O$_2$ (MH$^+$) 607.3755, found 607.3766. Anal. (C$_{37}$H$_{46}$N$_6$O$_2$) C, H, N.

Cyclopentyl (2S,3S)-3-Amino-1,4-diphenylbutan-2-ylcarbamate [(S,S)-16]. A solution of azide (S,S)-6a (510 mg, 1.35 mmol) in MeOH (14 mL) in a 100 mL round-bottom flask was treated with Pd(OH)$_2$ on carbon (98 mg of 20% w/w, 0.14 mmol, 10 mol %). The reaction flask was purged with H$_2$ and the reaction was allowed to stir under an atmosphere of H$_2$ for 2 h. The reaction mixture was filtered through a plug of Celite, washing and rinsing with MeOH; concentration under reduced pressure then gave the crude product. Purification by flushing through a short plug of silica with 5% MeOH in EtOAc removed the hydrazine byproduct and furnished the title compound as a white solid (470 mg, 99%; mp 85–87 °C). [α]$_D$ −16.6 (c 1.0, CDCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ = 7.29–7.12 (m, 10H), 5.27 (d, J = 8.4, 1H), 5.07–5.05 (m, 1H), 3.93 (br q, J = 7.2, 1H), 3.11–3.08 (m, 1H), 2.92 (dd, J = 13.8 and 7.2, 1H), 2.80 (dd, J = 13.8 and 4.2, 1H), 2.77 (dd, J = 13.8 and 7.8, 1H), 2.53 (dd, J = 13.2 and 9.6, 1H), 1.85–1.50 (m, 10H); ^{13}C NMR (150 MHz, CDCl$_3$) δ = 156.5, 138.7, 138.1, 129.2, 129.1, 128.5, 128.5, 126.4, 126.4, 77.3, 55.3, 53.0, 41.2, 39.4, 32.8, 32.7, 23.7, 23.6; HRMS (ESI-TOF) calculated for C$_{22}$H$_{29}$N$_2$O$_2$ (MH$^+$) 352.2223, found 353.2226.

Cyclopentyl (2S,3S)-3-[2-[4-(2,5-Dimethylphenyl)piperazin-1-yl]acetamido)-1,4-diphenylbutan-2-ylcarbamate [(S,S)-19a]. To a solution of amine (S,S)-16 (20 mg, 0.057 mmol, 1.0 equiv) in toluene (0.3 mL) was added water (0.3 mL) followed by triethanolamine (10 μL, 0.068 mmol, 1.2 equiv). The resulting biphasic reaction mixture was treated with chloroacetyl chloride (5.5 μL, 0.060 mmol, 1.05 equiv) and stirred at room temperature for 30 min. The reaction mixture was diluted with EtOAc (10 mL) and washed with H$_2$O (2 × 10 mL) followed by saturated NaHCO$_3$ solution (10 mL). The organic portion was dried over MgSO$_4$ and concentrated under reduced pressure to furnish (S,S)-17a (23 mg, 94%), which was used directly without further purification.

The α-chloro amide (S,S)-17a obtained as described above (23 mg, 0.054 mmol, 1.0 equiv) was dissolved in MeCN (0.54 mL) and treated with Hünig's base (11 μL, 0.065 mmol, 1.2 equiv) followed by 18 (10.3 mg, 0.054 mmol, 1.0 equiv). The reaction was stirred at room temperature for 20 h before being diluted with water (10 mL) and extracted with DCM (2 × 10 mL). The combined organic extracts were dried over MgSO$_4$ before being concentrated under reduced pressure to give the crude product. Purification by flash chromatography (45% EtOAc in hexanes) furnished the title compound as a white solid (24 mg, 75%; mp 180–182 °C). ^{1}H NMR (500 MHz, CDCl$_3$) δ = 7.32–6.82 (m, 14H), 5.18 (d, J = 8.0, 1H), 5.01 (br s, 1H), 4.23 (br s, 1H), 4.01 (br s, 1H), 3.06–2.77 (m, 10H), 2.46–2.34 (m, 4H), 2.34 (s, 3H), 2.22 (s, 3H), 1.82–1.53 (m, 8H); ^{13}C NMR (125 MHz, CDCl$_3$) δ = 170.9, 156.6, 151.0, 137.8, 136.1, 130.9, 129.4, 129.2, 129.0, 128.5, 128.4, 126.5, 126.5, 124.0, 119.7, 77.5, 61.5, 55.8, 53.8, 53.1, 51.7, 39.3, 38.2, 32.7, 32.6, 23.6, 23.5, 21.2, 17.3; HRMS (ESI-TOF) calculated for C$_{36}$H$_{47}$N$_4$O$_3$ (MH$^+$) 583.3642, found 583.3641. Anal. (C$_{36}$H$_{46}$N$_4$O$_3$) C, H, N.

Cyclopentyl (2*S*,3*S*)-3-(3-[4-(2,5-Dimethylphenyl)piperazin-1-yl]propanamido)-1,4-diphenylbutan-2-ylcarbamate [(*S*,*S*)-19b]. The same method was followed as for (*S*,*S*)-19a but using 3-bromopropionyl chloride in place of chloroacetyl chloride to furnish, after purification by flash chromatography (EtOAc), the title compound as a white solid (20 mg, 62% over two steps; mp 124−128 °C). ¹H NMR (500 MHz, CDCl₃) δ = 8.54 (d, J = 6.5, 1H), 7.26−7.16 (m, 10H), 7.06 (d, J = 7.5, 1H), 6.81 (d, J = 7.5, 1H), 6.75 (s, 1H), 5.63 (d, J = 8.5, 1H), 5.03−5.01 (m, 1H), 4.08−4.01 (m, 2H), 2.99−2.33 (m, 16H), 2.33 (s, 3H), 2.23 (s, 3H), 1.77−1.50 (m, 8H); ¹³C NMR (125 MHz, CDCl₃) δ = 173.1, 156.7, 150.7, 138.5, 138.0, 136.1, 130.9, 129.2, 129.1, 129.1, 128.4, 128.4, 126.4, 124.0, 119.6, 77.4, 55.5, 53.9, 53.5, 52.8, 51.6, 39.5, 37.9, 32.8, 32.6, 32.0, 23.6, 23.5, 21.2, 17.4; HRMS (ESI-TOF) calculated for C₃₇H₄₉N₄O₃ (MH⁺) 597.3805, found 597.3804. HPLC homogeneity 97.0% (system A), 95.3% (system B).

Cyclopentyl (2*S*,3*S*)-3-(2-[4-(2,5-Dimethylphenyl)piperazin-1-yl]-2-oxoethylamino)-1,4-diphenylbutan-2-ylcarbamate [(*S*,*S*)-20a]. To a solution of 18 (1.0 g, 5.3 mmol, 1.0 equiv) in toluene (20 mL) was added water (20 mL) followed by triethanolamine (0.84 mL, 6.3 mmol, 1.2 equiv). The resulting biphasic reaction mixture was treated dropwise with chloroacetyl chloride (0.44 mL, 5.5 mmol, 1.05 equiv) and stirred at room temperature for 5 h. The reaction mixture was diluted with EtOAc (50 mL) and washed with H₂O (3 × 30 mL). The organic portion was dried over MgSO₄ and concentrated under reduced pressure to furnish the α-chloro amide (1.4 g, quant), which was used directly without further purification.

The α-chloro amide obtained above (13 mg, 0.05 mmol, 1.0 equiv) was dissolved in MeCN (0.5 mL) and treated with Hünig's base (11 μL, 0.06 mmol, 1.2 equiv) followed by amine (*S*,*S*)-16 (18 mg, 0.05 mmol, 1.0 equiv) and a catalytic amount of TBAI. The reaction was heated to 55 °C for 64 h and then allowed to cool to room temperature before being diluted with water (10 mL) and extracted with DCM (2 × 10 mL). The combined organic extracts were dried over MgSO₄ before being concentrated under reduced pressure to give the crude product. Purification by flash chromatography (38% EtOAc in hexanes) furnished the title compound as a clear film (9 mg, 31%). ¹H NMR (600 MHz, CDCl₃) δ = 7.28−7.10 (m, 10H), 7.08 (d, J = 7.2, 1H), 6.84 (d, J = 7.2, 1H), 6.78 (s, 1H), 5.17 (d, J = 7.8, 1H), 5.03−5.01 (m, 1H), 3.99 (br q, J = 7.2, 1H), 3.80−3.71 (m, 2H), 3.49 (d, J = 15.6, 1H), 3.40−3.35 (m, 2H), 3.30 (d, J = 15.6, 1H), 2.89−2.74 (m, 9H), 2.31 (s, 3H), 2.27 (s, 3H), 1.84−1.48 (m, 9H); ¹³C NMR (125 MHz, CDCl₃) δ = 168.9, 156.4, 150.5, 138.7, 138.4, 136.3, 131.0, 129.4, 129.3, 129.1, 128.5, 128.3, 126.3, 126.2, 124.5, 119.9, 77.3, 61.2, 54.0, 51.7, 51.6, 49.8, 44.9, 42.5, 39.0, 38.8, 32.8, 32.6, 23.7, 23.6, 21.1, 17.3; HRMS (ESI-TOF) calculated for C₃₆H₄₇N₄O₃ (MH⁺) 583.3642, found 583.3639. HPLC homogeneity 93.0% (system A), 90.1% (system B).

Cyclopentyl (2*S*,3*S*)-3-(3-[4-(2,5-Dimethylphenyl)piperazin-1-yl]-3-oxopropylamino)-1,4-diphenylbutan-2-ylcarbamate [(*S*,*S*)-20b]. To a solution of 18 (1.0 g, 5.3 mmol, 1.0 equiv) in toluene (20 mL) was added water (20 mL) followed by triethanolamine (0.84 mL, 6.3 mmol, 1.2 equiv). The resulting biphasic reaction mixture was treated dropwise with 3-bromopropionyl chloride (0.59 mL, 5.5 mmol, 1.05 equiv) and stirred at room temperature for 30 min. The reaction mixture was diluted with EtOAc (50 mL) and washed with H₂O (2 × 40 mL) followed by saturated NaHCO₃ solution (40 mL). The organic portion was dried over MgSO₄ and concentrated under reduced pressure to furnish the β-bromo amide (1.7 g, quant), which was used directly without further purification.

The β-bromo amide obtained as described above (16 mg, 0.05 mmol, 1.0 equiv) was dissolved in MeCN (0.5 mL) and treated with Hünigs base (11 μL, 0.06 mmol, 1.2 equiv) followed by amine (*S*,*S*)-16 (18 mg, 0.05 mmol, 1.0 equiv) and a catalytic amount of TBAI. The reaction was heated to 55 °C for 24 h, a further portion of the β-bromo amide (64 mg, 0.2 mmol, 4.0 equiv) was then added, and the reaction was stirred for a further 72 h before being allowed

to cool to room temperature. The reaction mixture was diluted with water (10 mL) and extracted with DCM (2 × 10 mL). The combined organic extracts were dried over MgSO₄ before being concentrated under reduced pressure to give the crude product. Purification by flash chromatography (60−100% EtOAc in hexanes) furnished the title compound as a clear film (15 mg, 50%). ¹H NMR (500 MHz, CDCl₃) δ = 7.28−7.07 (m, 11H), 6.84 (d, J = 8.0, 1H), 6.79 (s, 1H), 5.15−5.02 (br m, 2H), 3.97−3.50 (br m, 6H), 2.99−2.39 (br m, 12H), 2.30 (s, 3H), 2.28 (s, 3H), 1.84−1.55 (m, 9H); ¹³C NMR (125 MHz, CDCl₃) δ = 170.2, 156.4, 150.6, 138.8, 138.4, 136.3, 131.0, 129.4, 129.3, 129.1, 128.4, 128.3, 126.2, 126.1, 124.4, 120.0, 77.1, 60.8, 53.9, 51.9, 51.6, 45.9, 44.7, 42.1, 39.1, 32.8, 32.6, 23.7, 23.6, 21.1, 17.4; HRMS (ESI-TOF) calculated for C₃₇H₄₉N₄O₃ (MH⁺) 596.3799, found 596.3798. HPLC homogeneity 95.0% (system A), 96.4% (system B).

Cyclopentyl (2*S*,3*S*)-3-[5-([4-(2,5-Dimethylphenyl)piperazin-1-yl]methyl)-1*H*-1,2,3-triazol-1-yl]-1,4-diphenylbutan-2-ylcarbamate [(*S*,*S*)-21]. A solution of azide (*S*,*S*)-6a (25 mg, 0.07 mmol, 1.0 equiv) and 1-(2,5-dimethylphenyl)-4-(prop-2-ynyl)piperazine (15 mg, 0.07 mmol, 1.0 equiv) in DMF (0.7 mL) was treated with (Cp*RuCl)₄ (3.6 mg, 0.003 mmol, 0.2 equiv of Ru). The reaction flask was flushed with N₂ and the reaction was heated to 50 °C for 16 h before being allowed to cool to room temperature. The reaction mixture was diluted with water (10 mL) and extracted with DCM (2 × 10 mL). The combined organic extracts were dried over MgSO₄ before being concentrated under reduced pressure to furnish the crude product. Purification by flash chromatography (30% EtOAc in hexanes) furnished the title compound as a pale solid (34 mg, 85%; mp 71−74 °C). ¹H NMR (500 MHz, CDCl₃): δ = 7.47 (s 1H), 7.30−6.90 (m, 11H), 6.79 (d, J = 7.5, 1H), 6.74 (s, 1H), 6.23 (d, J = 9.5, 1H), 5.04−4.99 (m, 1H), 4.75−4.71 (m, 1H), 4.67−4.61 (m, 1H), 3.36−3.28 (m, 2H), 2.80−2.30 (m, 12H), 2.28 (s, 3H), 2.19 (s, 3H), 1.81−1.54 (m, 8H); ¹³C NMR (125 MHz, CDCl₃) δ = 156.2, 150.8, 137.2, 137.1, 136.1, 135.2, 133.2, 130.8, 129.2, 129.1, 129.0, 128.6, 128.5, 127.0, 126.6, 123.9, 119.6, 77.5, 62.8, 54.3, 53.6, 51.4, 50.0, 39.5, 38.6, 32.8, 32.6, 23.6, 23.5, 21.1, 17.3; HRMS (ESI-TOF) calculated for C₃₇H₄₇N₆O₂ (MH⁺) 607.3755, found 607.3759. HPLC homogeneity 94.1% (system A), 90.7% (system B).

Cyclopentyl (2*S*,3*S*)-3-[4-([4-(2,5-Dimethylphenyl)piperazin-1-yl]methyl)-5-(trimethylsilyl)-1*H*-1,2,3-triazol-1-yl]-1,4-diphenylbutan-2-ylcarbamate [(*S*,*S*)-22a]. A solution of triazole (*S*,*S*)-11k (20 mg, 0.033 mmol, 1.0 equiv) in THF (0.7 mL) was cooled to −78 °C before being treated dropwise with *n*-BuLi (38 μL of a 1.9 M solution in hexanes, 0.073 mmol, 2.2 equiv). The solution was allowed to stir for 5 min at −78 °C before being treated with TMS-Cl and allowed to warm to room temperature. After being allowed to stir for 30 min at room temperature, the reaction was quenched by addition of water (5 mL) and extracted with DCM (2 × 10 mL). The combined organic extracts were dried over MgSO₄ before being concentrated under reduced pressure to furnish the crude product. Purification by flash chromatography (15−20% EtOAc in hexanes) furnished the title compound as a pale film (13 mg, 58%). ¹H NMR (600 MHz, CDCl₃) δ = 7.26−6.73 (m, 13H), 6.32 (d, J = 10.2, 1H), 5.01−4.99 (m, 1H), 4.66−4.60 (m, 2H), 3.72 (d, J = 12.6, 1H), 3.66 (d, J = 12.6, 1H), 3.47 (dd, J = 13.8 and 10.2, 1H), 3.16 (dd, J = 13.8 and 3.6, 1H), 2.92−2.51 (br m, 8H), 2.47 (dd, J = 13.8 and 6.6, 1H), 2.39 (dd, J = 13.8 and 8.4, 1H), 2.30 (s, 3H), 2.27 (s, 3H), 1.80−1.50 (m, 8H), −0.07 (s, 9H); ¹³C NMR (150 MHz, CDCl₃) δ = 156.4, 151.3, 151.1, 137.1, 136.8, 136.0, 135.5, 130.9, 129.3, 129.1, 128.6, 128.6, 127.0, 126.7, 123.6, 119.5, 77.5, 63.9, 54.9, 54.3, 53.4, 51.5, 40.4, 38.3, 32.7, 32.6 23.6, 23.5, 21.2, 17.5, −0.5; HRMS (ESI-TOF) calculated for C₄₀H₅₅N₆O₂Si (MH⁺) 679.4156, found 679.4146. HPLC homogeneity 95.5% (system A), 94.7% (system B).

Cyclopentyl (2*S*,3*S*)-3-[4-([4-(2,5-Dimethylphenyl)piperazin-1-yl]methyl)-5-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl]-1,4-diphenylbutan-2-ylcarbamate [(*S*,*S*)-22b]. A solution of triazole (*S*,*S*)-11k (50 mg, 0.083 mmol, 1.0 equiv) in THF (1.5 mL) was cooled

to −78 °C before being treated dropwise with *n*-BuLi (85 μL of a 2.5 M solution in hexanes, 0.21 mmol, 2.5 equiv). The solution was allowed to stir for 5 min at −78 °C before being transferred by cannula onto *p*-CH₂O (25 mg, 0.83 mmol, 10 equiv) at 0 °C. The reaction rapidly turned orange, then brown over 20 min, and then again became colorless after 40 min. After being allowed to stir for a further 20 min, the reaction was quenched by addition of water (5 mL) and extracted with DCM (2 × 15 mL). The combined organic layers were dried over MgSO₄ before being concentrated under reduced pressure to furnish the crude product. Purification by preparative TLC (70% EtOAc in hexanes) furnished the title compound as a clear film (28 mg, 53%). ¹H NMR (600 MHz, C₆D₆) δ = 7.17−6.39 (m, 14H), 5.32−5.30 (m, 1H), 4.81−4.77 (m, 1H), 4.17−4.12 (m, 1H), 3.88 (d, *J* = 14.4, 1H), 3.67 (d, *J* = 13.2, 1H), 3.56 (d, *J* = 13.2, 1H), 3.43 (d, *J* = 14.4, 1H), 3.33, (dd, *J* = 13.8 and 11.8, 1H), 2.94 (dd, *J* = 13.8 and 3.6, 1H), 2.90 (dd, *J* = 13.8 and 6.6, 1H), 2.71−2.57 (m, 4H), 2.42 (dd, *J* = 13.8 and 9.0, 1H), 2.34−2.21 (m, 4H), 2.20 (s, 3H), 2.14 (s, 3H), 1.80−1.29 (m, 9H); ¹³C NMR (150 MHz, C₆D₆) 156.7, 151.3, 141.8, 137.9, 137.6, 137.2, 136.2, 131.2, 129.7, 129.4, 129.3, 129.0, 128.3, 127.1, 126.9, 124.6, 120.3, 77.8, 66.2, 56.2, 54.3, 54.2, 53.2, 51.7, 40.2, 39.6, 33.1, 33.0, 24.0, 23.9, 21.2, 17.6; HRMS (ESI-TOF) calculated for C₃₈H₄₉N₆O₃ (MH⁺) 637.3866, found 637.3864. HPLC homogeneity 97.4% (system A), 96.9% (system B).

Cyclopentyl (2S,3S)-3-[4-([4-(2,5-Dimethylphenyl)piperazin-1-yl]methyl)-5-[(S)-1-hydroxyethyl]-1H-1,2,3-triazol-1-yl]-1,4-diphenylbutan-2-ylcarbamate [(S,S,S)-22c] and Cyclopentyl (2S,3S)-3-[4-([4-(2,5-Dimethylphenyl)piperazin-1-yl]methyl)-5-[(R)-1-hydroxyethyl]-1H-1,2,3-triazol-1-yl]-1,4-diphenylbutan-2-ylcarbamate [(S,S,R)-22c]. A solution of triazole (S,S)-11k (30 mg, 0.05 mmol, 1.0 equiv) in THF (1 mL) was cooled to −78 °C before being treated dropwise with *n*-BuLi (58 μL of a 1.9 M solution in hexanes, 0.11 mmol, 2.2 equiv). The solution was allowed to stir for 5 min at −78 °C before being treated with acetaldehyde (8.5 μL, 0.15 mmol 3.0 equiv). The reaction was maintained at −78 °C for 5 min and then allowed to warm to room temperature. After being allowed to stir for a further 30 min, the reaction was quenched by addition of water (5 mL) and extracted with DCM (2 × 15 mL). The combined organic extracts were dried over MgSO₄ before being concentrated under reduced pressure to furnish the crude product, which ¹H NMR indicated was an approximately 3:1 mixture of diastereomers. Purification by flash chromatography (30−50% EtOAc in hexanes) enabled separation of both the major, least polar S,S,S isomer as a white solid (21 mg, 65%; mp 193−195 °C) and the minor, more polar S,S,R isomer contaminated with an unknown impurity. Preparative TLC (8% MeOH in DCM) of the minor product enabled isolation of the pure compound (7.5 mg, 23%) as a clear film The configuration of the newly formed stereogenic center was assigned by analogy to that of (S,S,S)-23c, which was confirmed by the acquisition of an X-ray crystal structure.[16]

Data for major, least polar S,S,S isomer: ¹H NMR (600 MHz, C₆D₆) δ = 7.31 (d, *J* = 7.2, 2H), 7.21 (t, *J* = 7.2, 2H), 7.04−7.00 (m, 2H), 6.87−6.85 (m, 3H), 6.76 (d, *J* = 7.2, 1H), 6.64−6.61 (m, 2H), 6.52−6.50 (m, 2H), 5.33−5.30 (m, 1H), 4.77−4.72 (m, 1H), 4.35 (dt, *J* = 11.4 and 3.6, 1H), 4.30 (q, *J* = 6.6, 1H), 3.64 (d, *J* = 13.2, 1H), 3.57 (d, *J* = 13.2, 1H), 3.53 (dd, *J* = 13.8 and 11.4, 1H), 2.97 (dd, *J* = 13.8 and 3.6, 1H), 2.80 (dd, *J* = 13.8 and 6.6, 1H), 2.70−2.63 (m, 4H), 2.43 (dd, *J* = 13.8 and 8.4, 1H), 2.41−2.26 (m, 4H), 2.17 (s, 3H), 2.11 (s, 3H), 1.82−1.28 (m, 9H), 0.61 (d, *J* = 6.6, 3H); ¹³C NMR (150 MHz, C₆D₆) δ = 156.6, 151.2, 142.0, 140.9, 137.8, 137.5, 136.2, 131.2, 129.7, 129.6, 129.4, 129.1, 128.8, 127.1, 127.0, 124.5, 120.3, 77.8, 62.3, 60.8, 57.1, 54.6, 53.3 (b), 51.7, 40.1, 39.8, 33.1, 33.0, 24.0, 23.9, 23.0, 21.2, 17.5; HRMS (ESI-TOF) calculated for C₃₉H₅₁N₆O₃ (MH⁺) 651.4023, found 651.4024. HPLC homogeneity 97.0% (system A), 95.4% (system B).

Data for minor, more polar S,S,R isomer: ¹H NMR (600 MHz, C₆D₆) δ = 7.11 (t, *J* = 7.2, 2H), 7.06−7.01 (m, 4H), 6.94 (t, *J* = 7.2, 2H), 6.88 (t, *J* = 7.2, 1H), 6.79 (d, *J* = 7.8, 1H), 6.76 (d, *J* =

7.2, 2H), 6.71 (s, 1H), 6.13 (d, *J* = 9.6, 1H), 5.18−5.14 (m, 1H), 4.75−4.69 (m, 1H), 4.45 (dt, *J* = 10.2 and 4.2, 1H), 4.28 (q, *J* = 6.6, 1H), 3.67 (d, *J* = 13.2, 1H), 3.61 (d, *J* = 13.2, 1H), 3.42 (dd, *J* = 13.8 and 12.0, 1H), 3.14 (dd, *J* = 13.8 and 4.8, 1H), 2.77−2.71 (m, 2H), 2.70 (dd, *J* = 13.8 and 5.4, 1H), 2.63−2.59 (m, 2H), 2.51 (dd, *J* = 13.8 and 10.2, 1H), 2.29 (br s, 4H), 2.24 (s, 3H), 2.16 (s, 3H), 1.74−1.50 (m, 7H), 0.1.31−1.25 (m, 2H), 1.26 (d, *J* = 6.6, 3H); ¹³C NMR (150 MHz, C₆D₆) δ = 156.6, 151.4, 141.4, 140.6, 137.7, 137.0, 136.2, 131.2, 129.7, 129.5, 129.3, 128.8, 128.7, 127.0, 126.8, 124.6, 120.4, 77.7, 63.4, 61.1, 55.3, 54.4, 53.3 (b), 51.8, 40.0, 39.0, 33.0, 32.9 24.2, 23.9, 23.8, 21.2, 17.6; HRMS (ESI-TOF) calculated for C₃₉H₅₁N₆O₃ (MH⁺) 651.4023, found 651.4018. HPLC homogeneity 96.2% (system A), 96.0% (system B).

Cyclopentyl (2S,3S)-3-[4-([4-(2,5-Dimethylphenyl)piperazin-1-yl]methyl)-5-[(S)-1-hydroxypropyl]-1H-1,2,3-triazol-1-yl]-1,4-diphenylbutan-2-ylcarbamate [(S,S,S)-22d] and Cyclopentyl (2S,3S)-3-[4-([4-(2,5-Dimethylphenyl)piperazin-1-yl]methyl)-5-[(R)-1-hydroxypropyl]-1H-1,2,3-triazol-1-yl]-1,4-diphenylbutan-2-ylcarbamate [(S,S,R)-22d]. A solution of triazole (S,S)-11k (60 mg, 0.10 mmol, 1.0 equiv) in THF (2 mL) was cooled to −78 °C before being treated dropwise with *n*-BuLi (116 μL of a 1.9 M solution in hexanes, 0.22 mmol, 2.2 equiv). The solution was allowed to stir for 5 min at −78 °C before being treated with propionaldehyde (22 μL, 0.30 mmol 3.0 equiv). The reaction was maintained at −78 °C for 5 min and then allowed to warm to room temperature. After being allowed to stir for a further 30 min, the reaction was quenched by addition of water (5 mL) and extracted with DCM (2 × 15 mL). The combined organics were dried over MgSO₄ before being concentrated under reduced pressure to furnish the crude product, which ¹H NMR indicated was an approximately 3:1 mixture of diastereomers. Purification by preparative TLC (45% EtOAc in hexanes) enabled separation of both the major, least polar S,S,S isomer (30 mg, 45%) and the minor, more polar S,S,R isomer (10 mg, 15%) as clear films.

Data for major, least polar S,S,S isomer: ¹H NMR (600 MHz, C₆D₆) δ = 7.31−6.55 (m, 14H), 5.33−5.30 (m, 1H), 4.78−4.72 (m, 1H), 4.39 (dt, *J* = 10.8 and 3.6, 1H), 4.06 (dd, *J* = 9.6 and 3.6, 1H), 3.64 (d, *J* = 13.2, 1H), 3.58 (d, *J* = 13.2, 1H), 3.55 (dd, *J* = 13.8 and 10.8, 1H), 3.03 (dd, *J* = 13.8 and 3.6, 1H), 2.75 (dd, *J* = 13.8 and 6.6, 1H), 2.71−2.65 (m, 4H), 2.45 (dd, *J* = 13.8 and 8.4, 1H), 2.43−2.24 (m, 4H), 2.18 (s, 3H), 2.12 (s, 3H), 1.81−1.17 (m, 11H), 0.77 (t, *J* = 7.2, 3H); ¹³C NMR (150 MHz, C₆D₆) 156.6, 151.2, 141.4, 141.2, 137.8, 137.6, 136.2, 131.1, 129.6, 129.5, 129.4, 129.0, 128.8, 127.1, 126.9, 124.5, 120.3, 77.8, 66.2, 62.3, 57.0, 54.5, 51.6, 39.9, 39.6, 33.1, 33.0, 30.2, 24.0, 23.9, 21.1, 17.9, 11.1; HRMS (ESI-TOF) calculated for C₄₀H₅₃N₆O₃ (MH⁺) 665.4179, found 665.4178. HPLC homogeneity 100% (system A), 95.3% (system B).

Data for minor, most polar S,S,R isomer: ¹H NMR (600 MHz, C₆D₆) δ = 7.29−6.72 (m, 13H), 6.06 (d, *J* = 9.6, 1H), 5.19−5.16 (m, 1H), 4.76−4.71 (m, 1H), 5.13 (dt, *J* = 9.6 and 4.8, 1H), 4.11 (dd, *J* = 7.8 and 3.6, 1H), 3.65 (d, *J* = 13.2, 1H), 3.62 (d, *J* = 13.2, 1H), 3.41 (dd, *J* = 13.8 and 10.8, 1H), 3.13 (dd, *J* = 13.8 and 4.8, 1H), 2.77−2.54 (m, 6H), 2.29 (br s, 4H), 2.26 (s, 3H), 2.17 (s, 3H), 1.74−1.26 (m, 11H), 0.99 (t, *J* = 7.2, 3H); ¹³C NMR (150 MHz, C₆D₆) 156.5, 151.5, 141.0, 140.6, 137.8, 136.9, 136.2, 131.2, 129.7, 129.4, 129.3, 128.8, 128.7, 127.0, 126.8, 124.6, 120.4, 77.7, 66.3, 63.3, 55.3, 54.6, 51.8, 40.0, 38.8, 33.0, 32.9, 31.1, 23.9, 23.8, 21.3, 17.6, 10.4; HRMS (ESI-TOF) calculated for C₄₀H₅₃N₆O₃ (MH⁺) 665.4179, found 665.4175. HPLC homogeneity 100% (system A), 100% (system B).

Cyclopentyl (2S,3S)-3-[4-([4-(5-Chloro-2-methylphenyl)piperazin-1-yl]methyl)-5-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]-1,4-diphenylbutan-2-ylcarbamate (S,S)-24b. The same method was followed as for the synthesis of (S,S)-22b but starting from triazole (S,S)-11j in place of triazole (S,S)-11k. Purification of the crude product by preparative TLC on neutral alumina (50% EtOAc in hexanes) furnished the title compound as a pale solid (58%; mp 85−87 °C). ¹H NMR (600 MHz, C₆D₆) δ = 7.15 (br s, 2H), 7.04−

7.00 (m, 3H), 6.91 (dd, $J = 8.1$ and 1.8, 1H), 6.82 (d, $J = 1.8$, 1H), 6.79−6.74 (m, 4H), 6.62 (br d, $J = 9.6$, 1H), 6.38 (br d, $J = 6.6$ Hz, 2H), 5.33−5.32 (m, 1H), 4.80−4.79 (m, 1H), 4.13 (br d, $J = 11.4$, 1H), 3.86 (d, $J = 15.0$, 1H), 3.62 (d, $J = 13.2$, 1H), 3.52 (d, $J = 13.2$, 1H), 3.41 (d, $J = 14.4$, 1H), 3.30 (dd, $J = 13.2$ and 12.0, 1H), 2.91 (td, $J = 13.2$ and 3.6, 2H), 2.45−2.40 (m, 5H), 2.15 (br s, 4H), 1.98 (s, 3H), 1.80−1.78 (m, 1H), 1.71−1.68 (m, 3H), 1.60−1.58 (m, 2H), 1.35−1.30 (m, 3H); ^{13}C NMR (150 MHz, C_6D_6) $\delta = 156.7$, 152.4, 138.0, 137.5, 137.2, 132.3, 131.0, 129.4, 129.3, 129.0, 128.6, 128.3, 127.2, 126.9, 123.7, 120.1, 77.9, 62.2, 56.2, 54.2, 52.9, 51.1, 40.2, 39.6, 33.1, 33.0, 24.0, 23.9, 17.4; HRMS (ESI-TOF) calculated for $C_{37}H_{46}ClN_6O_3$ (MH$^+$) 657.3314, found 657.3311. HPLC homogeneity 95.5% (system A), 97.0% (system B).

Supporting Information Available: Elemental analysis results and HPLC homogeneity data for selected products; details of library synthesis; synthetic procedures and characterization data for compounds (*S,S*)-**7e**, (*S,S*)-**15a**, (*S,S*)-**15e**, (*S,S,S*)-**24c**, and (*S,S,R*)-**24c**; and synthesis details, characterization, and X-ray crystallographic data for compound (*S,S*)-**23c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Synthesis of Saccharide-Terminated Poly(ε-caprolactone) via Michael Addition and 'Click' Chemistry[a]

Ning Xu, Feng-Zhu Lu, Fu-Sheng Du, Zi-Chen Li*

Maleimido-terminated PCL (M-PCL) and alkyne-terminated PCL (A-PCL) are prepared by the ring-opening polymerization of ε-caprolactone with N-hydroxyethyl maleimide and 4-pentyn-1-ol as initiators catalyzed by tin(II) trifluoromethane sulfonate at 25 °C, respectively. A series of saccharide-terminated PCLs have also been synthesized under mild conditions by two chemical strategies: 1). Michael addition of M-PCL and amino-containing maltose, and 2). a 'click' reaction of A-PCL and azide-containing saccharide. The amphiphilic nature of these maltose-terminated PCLs make self-assembly into aggregates in water possible. These aggregates have been characterized by transmission electron microscopy and dynamic light scattering measurements.
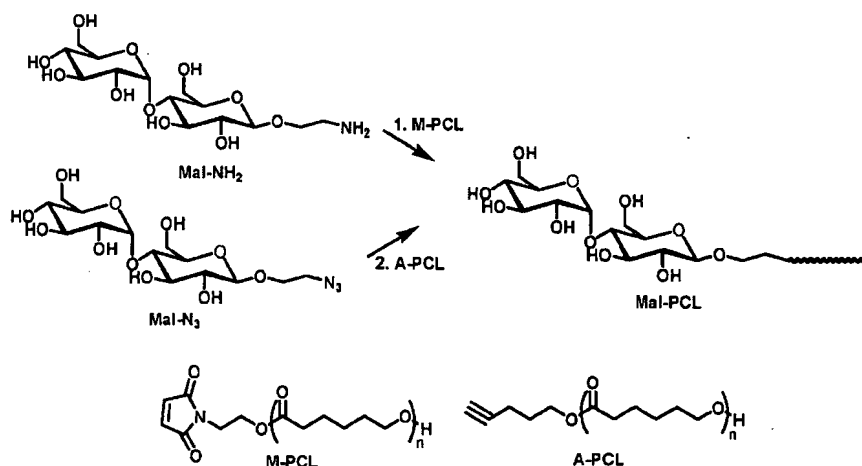
N. Xu, F.-Z. Lu, F.-S. Du, Z.-C. Li
Key Laboratory of Polymer Chemistry & Physics, Ministry of Education of China, Department of Polymer Science & Engineering, College of Chemistry, Peking University, Beijing 100871, China
Fax: +86 10 6275 1708; E-mail: zcli@pku.edu.cn

[a] Supporting information for this article is available at the bottom of the article's abstract page, which can be accessed from the journal's homepage at http://www.mcp-journal.de, or from the author.

Introduction

Aliphatic polyesters, especially those based on ε-caprolactone (CL), lactide (LA), and glycolide, have been widely used in biomedical fields.[1] These polyesters are usually hydrophobic and semicrystalline, with no functional groups on the polymer backbone. Therefore, their applications are limited, and the introduction of functional groups or hydrophilic segments either to the chain end or as pendent groups to these aliphatic polyesters has gained

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increasing attention.[2] So far, numerous successful examples have been reported, including the chain-end functionalized aliphatic polyesters with thiol-, amino-, carboxy-, phosphoryl choline, and saccharide groups, or poly(ethylene oxide) blocks through post modification of polyesters or ring-opening polymerization (ROP) initiated by functional groups that contain initiators.[2,3] Aliphatic polyesters with pendent functional groups have also been synthesized by ROP of functionalized lactones, lactides, or through post-polymerization modification. In the former case, the functional groups should be protected before polymerization, while in the latter case, the reaction conditions must be chosen carefully to avoid any degradation or cross-linking of both backbone and side chains.[2,4]

Saccharides are important in nature because of their important role in many biological recognition processes on cellular surfaces.[5] Modification of biodegradable polyesters with saccharides, with an aim to improve the solubility in water and increase the functions of the polyesters, has been an active research field in recent years.[6–8] The synthesis of saccharide end-capped polyesters can be achieved either by the ROP of lactones and lactides initiated by saccharides, or through the chemical reaction between saccharides and polyesters. Since saccharides contain many hydroxy groups, initiation with saccharides needs many protecting-deprotecting steps. Post-polymerization modification is, therefore, a desirable alternative, provided an efficient reaction is used under mild conditions. Saccharide-terminated poly(ε-caprolactone) (PCL) prepared by this approach has a clear and controlled structure. In addition, unprotected saccharides can be used directly to prepare PCL with the same degree of polymerization (DP) and different saccharide end groups.

The Michael addition of maleimide and amino groups has been widely used in organic synthesis because of its efficiency and high selectivity. Recently, this reaction became an important method to modify polymers.[9] 'Click' chemistry, originally developed for use in organic synthesis and chemical biology, is highly efficient and selective, and can be carried out in the presence of many functional groups with high yields.[10] Recently, this 1,3-dipolar cycloaddition of azides and terminated alkynes by copper(ı) catalyst has been widely used in the synthesis of end- or pendent functionalized polymers,[11] new monomers and macromonomers,[12] block copolymers, star, or cyclic polymers,[13] and dendrimers.[14] As this method is efficient for both biomaterials[15] and polymers, a new strategy has recently been provided to synthesize glycopolymers.[16]

In this paper, two kinds of end-functionalized PCL have been initially synthesized, and maltose is conjugated to them by a Michael addition reaction and a click reaction, respectively. In the click reaction, a triethoxy group has been chosen as a spacer between the azide and saccharide to increase the hydrophilicity of the polyester. The advantage of these two strategies is that hydrophilic saccharide groups can be efficiently and directly conjugated to the polyesters without a protection-deprotection procedure. Although the click reaction has been used to prepare small molecule saccharide derivatives, glycopolymers,[10a,14f,16,17] and PCL with pendent groups,[4c,11f] to the best of our knowledge, this is the first report on the synthesis of PCL terminated by saccharides using a Michael addition reaction and click chemistry. The saccharide-terminated PCL are expected to find applications in the field of drug-delivery systems.

Experimental Part

Materials

CL (Acros, 99%) was dried over CaH_2 at room temperature for 48 h, and then distilled under reduced pressure just before use. Tin(ıı) trifluoromethane sulfonate ($Sn(OTf)_2$, Alfa Aeser, 97%) was dissolved in toluene/tetrahydrofuran (THF) (80:20, v/v) at a concentration of $15 \text{ mg} \cdot \text{mL}^{-1}$. Pd/C (10 wt.-%) and *Concanavalin A* (Con A) was from Sigma. Copper(ı) bromide ($Cu^I Br = 99.999\%$), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, 99%), and 4-pentyn-1-ol (95%) were purchased from Aldrich. *N*-Hydroxyethyl maleimide (HEMI) was synthesized according to the literature method.[18] 2-Azidoethoxyl-β-ᴅ-glucopyranoside (Glu-N_3), 2-azidoethoxyl 4-*O*-[2,3,4,6-tetra-*O*-benzyl-α-ᴅ-glucopyranosyl]-2,3,6-tri-*O*-benzyl-β-ᴅ-glucopyranoside (BnMal-N_3), 2-azidoethoxy-4-*O*-[α-ᴅ-glucopyranosyl]-β-ᴅ-glucopyranoside (Mal-N_3), 2-aminoethoxy-4-*O*-[α-ᴅ-glucopyranosyl]-β-ᴅ-glucopyranoside (Mal-NH_2), and 2-[2-(2-azidoethoxy)ethoxy]ethoxy-4-*O*-[α-ᴅ-glucopyranosyl]-β-ᴅ-glucopyranoside (TEMal-N_3) were synthesized according to the literature procedures.[19,20]

Synthesis of Maleimido-Terminated PCL (M-PCL)

A typical procedure for the synthesis of M-PCL_{10} is described as follows: CL (5.00 g, 44 mmol) and an $Sn(OTf)_2$ solution ($15 \text{ mg} \cdot \text{mL}^{-1}$, 100 µL) were placed in a tube and stirred for 20 min before HEMI (0.60 g, 4.4 mmol) was added. The tube was then subjected to two freeze-pump-thaw cycles and then sealed under vacuum. The mixture was stirred at room temperature for 48 h. Afterwards, the mixture was dissolved in $CHCl_3$ before precipitation into cold methanol. The precipitate was collected as M-PCL_{10} and dried under vacuum with a 94% yield. M-PCL_{17} and M-PCL_{22} were prepared in a similar manner by changing the molar ratio of CL to HEMI.

Synthesis of Alkyne-Terminated PCL (A-PCL)

The synthetic procedure was similar to that for the preparation of M-PCL except that 4-pentyn-1-ol was used as the initiator. A-PCL_{17} and A-PCL_{35} were prepared with different ratios of monomer to initiator.

Synthesis of Maltose-Terminated PCL (Mal-PCL) by Michael Addition

A typical procedure for the synthesis of Mal-PCL_{17} is described as follows: M-PCL_{17} (0.29 g, 0.14 mmol of maleimido residue),

Mal-NH$_2$ (77 mg, 0.21 mmol), and 4 mL of DMSO were placed in a round-bottom flask and stirred for 48 h at room temperature. The reaction mixture was then precipitated in H$_2$O to completely remove excess Mal-NH$_2$. The precipitate was dissolved in CHCl$_3$ and re-precipitated in methanol. The desired product was obtained by filtration as a white powder. Mal-PCL$_{10}$ and Mal-PCL$_{22}$ were prepared in a similar way.

Synthesis of Mal-PCL by Click Chemistry

A typical procedure for the synthesis of Mal-PCL$_{17}$ is described as follows: A-PCL$_{17}$ (0.49 g, 0.24 mmol of alkyne group), Mal-N$_3$ (0.25 g, 0.36 mmol), 5 mL of acetone, and 26 μL of PMDETA were charged to a tube and stirred for 15 min. The tube was then subjected to two freeze-pump-thaw cycles. Cu^{I}Br (17 mg) was added quickly, and the tube was then sealed under vacuum and placed into an oil bath. The mixture was stirred for 48 h. The tube was broken and the mixture was precipitated into methanol twice. Products were dried under vacuum.

Mal-PCL can also be prepared from the click chemistry of BnMal-N$_3$ and A-PCL, followed by deprotection of the protective groups. A typical procedure for the synthesis of BnMal-PCL$_{17}$ is described as follows: A-PCL$_{17}$ (0.49 g, 0.24 mmol of alkyne group), BnMal-N$_3$ (0.49 g, 0.48 mmol), solvent, and ligand were charged to a tube and stirred for 15 min. The tube was then subjected to two freeze-pump-thaw cycles. Cu^{I}Br was added quickly, and then the tube was sealed under vacuum and placed into a pre-heated oil bath, the mixture was stirred for a desired time. The mixture was then diluted with THF and passed through a short column of neutral alumina. The solution was concentrated and precipitated into methanol to completely remove excess BnMal-N$_3$. The product was obtained by filtration as a white powder. BnMal-PCL$_{35}$ was prepared in a similar way.

The benzyl protecting groups of BnMal-PCL were removed by catalytic hydrogenolysis. BnMal-PCL (0.20 g) and Pd/C (20 mg) were charged into a round-bottom flask and 12 mL of EtOAc/methanol (2:1, v/v) was added. Hydrogen was introduced to the system and the mixture was stirred for 72 h at room temperature under H$_2$ atmosphere. Pd/C powder was removed by filtration on a short column of Celite, the filtrate was concentrated and pre-

cipitated into cold Et$_2$O. A pale yellow powder was obtained in 67% yield.

Synthesis of Glucose-Terminated PCL$_{17}$ (Glu-PCL$_{17}$) by Click Chemistry

The synthetic procedure for the synthesis of Glu-PCL$_{17}$ was similar to that of Mal-PCL from Glu-N$_3$ and A-PCL$_{17}$ in 85% yield.

Synthesis of TEMal-Terminated PCL (TEMal-PCL)

The synthetic procedure was similar to that of Mal-PCL, and the starting materials were TEMal-N$_3$ and A-PCL.

Scheme 1. Synthesis of Mal-PCL (a) and A-PCL (b).

Instrumentation

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AXR-400 MHz or a Varian 300 MHz spectrometer, CDCl$_3$, D$_2$O, acetone-d_6, and dimethyl sulfoxide-d_6 (DMSO) were used as solvents. FT-IR spectra were recorded in KBr pellets using a VECTOR 22 FT-IR spectrometer. Average molecular weights ($\overline{M}_n$) and polydispersities (PDIs) of the polymers were measured by gel permeation chromatography (GPC) equipped with a Waters 410 refractive index detector, a Water 515 HPLC pump, and Ultrastyragel columns (10^4, 10^3 and 500 Å) at 35 °C with THF as eluent at a flow rate of 1 mL·min^{-1}. Monodisperse polystyrene standards were used as calibrants.

Table 1. Synthesis of end-functionalized PCL in bulk.

Sample[a]	Initiator	M/I[b]	DP[c]	$\overline{M}_n$ [d]	$\overline{M}_w/\overline{M}_n$ [d]	Yield[e] %
M-PCL$_{10}$	HEMI	10	10	2 800	1.09	94
M-PCL$_{17}$	HEMI	15	17	2 900	1.13	99
M-PCL$_{22}$	HEMI	25	22	3 300	1.13	59
A-PCL$_{10}$	4-pentyn-1-ol	10	10	2 500	1.13	84
A-PCL$_{17}$	4-pentyn-1-ol	20	17	3 000	1.13	94
A-PCL$_{35}$	4-pentyn-1-ol	40	35	6 700	1.13	90

[a]The polymerizations were catalyzed by Sn(OTf)$_2$ at a concentration of 15 mg·mL^{-1} in THF/toluene (1:4, v/v) at 25 °C for 48 h; [b]Molar ratio of monomer to initiator; [c]Degree of polymerization calculated from ^{1}H NMR spectroscopy; [d]Determined by GPC; [e]Determined gravimetrically.

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Polymer Aggregates

Preparation and TEM Observation of the Aggregates

TEMal-PCL$_{10}$ and TEMal-PCL$_{17}$ aggregate suspension samples were prepared by dissolving the solid samples directly in water at a concentration of 0.5 mg·mL^{-1}. One drop of the aggregate suspension was placed onto a copper mesh covered with Formvar membrane for 30 min followed by negative staining with uranyl acetate for 1 min (saturated solution in 95% alcohol). The morphology was observed on a JEOL JEM-100CXII transmission electron microscope (TEM) at an acceleration voltage of 80 kV.

Dynamic Light Scattering (DLS) Measurement of the Aggregates

Samples of the polymer aggregate suspension were obtained by diluting the samples for TEM observation. Mean diameters of the prepared polymer aggregates in water (0.1 mg·mL^{-1}) were determined by DLS using an AVL/DLS/SLS-5022F instrument at 25 °C at a detection angle of 90°.

Interaction of the Aggregates with Con A

Polymer aggregate suspensions were prepared by dissolving TEMal-PCL$_{10}$ in a buffer solution (pH = 7.2, 0.10 M Tris·HCl, 0.9 M NaCl, 1 × 10^{-3} M CaCl$_2$, and 1 × 10^{-3} M MnCl$_2$) at a concentration of 0.5 mg·mL^{-1}. Con A was dissolved in the same buffer solution at a concentration of 2 mg·mL^{-1}. Equal volumes of the two solutions were mixed and the transmittance at 500 nm was followed on a
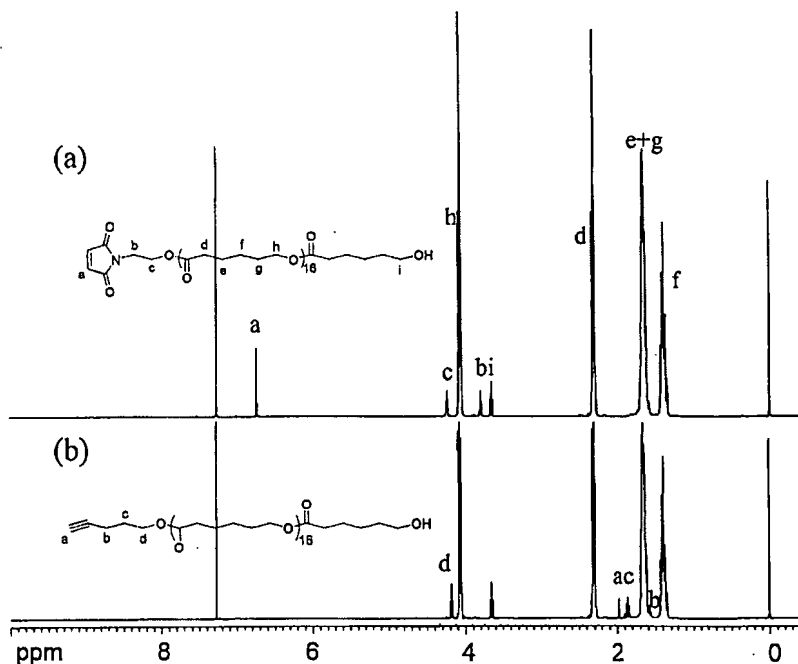
Figure 1. ^{1}H NMR spectra of Mal-PCL$_{17}$ (a) and A-PCL$_{17}$ (b) in CDCl$_3$.

UV-2101 UV-vis spectrometer. Con A solutions were measured as a control.

Results and Discussion

Synthesis of End-Functionalized PCL

End-functionalized PCL can be synthesized by the ROP of CL catalyzed by lipase[21] or metal catalysts.[22] Tin-containing
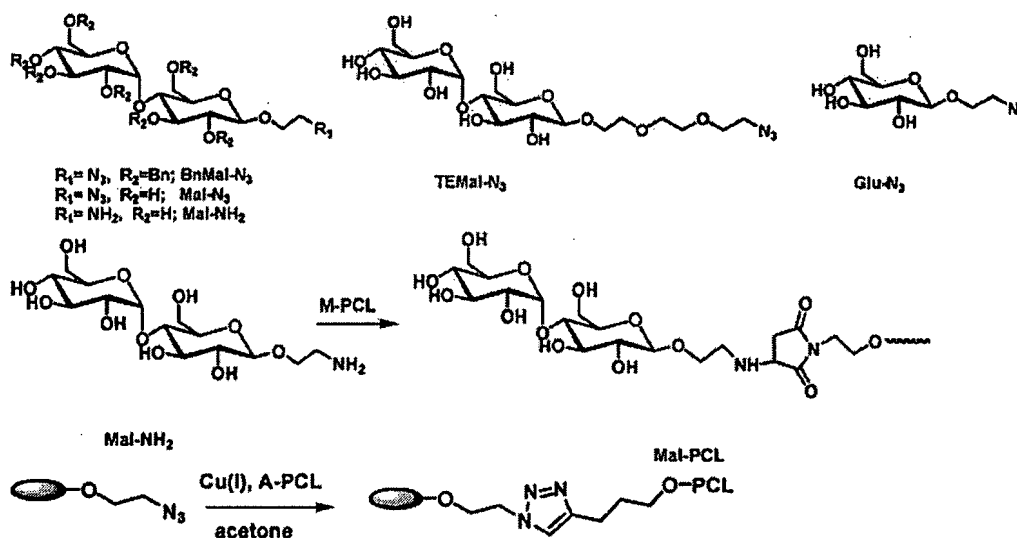
Scheme 2. Structures of saccharide derivatives and the synthesis of Mal-PCL by Michael addition or click chemistry.

catalysts are often used together with hydro-xy-containing functional initiators. Depending on the type of catalysts, the polymerization can be carried out in bulk or in solution, at high or low temperature. In this paper, $Sn(OTf)_2$ was used as the catalyst, which could catalyze the polymerization of CL at room temperature.[23] M-PCL and A-PCL were prepared at room temperature (25 °C) in solution for 48 h, with HEMI and 4-pentyn-1-ol as initiators, respectively (Scheme 1).

Polymerization data are summarized in Table 1. The DP of the M-PCL was calculated from the integration ratio of the signals at $\delta = 2.0$ ppm ($-OC(O)CH_2-$) and $\delta = 6.8$ ($-CH=CH-$) in the ^1H NMR spectrum (Figure 1a). Similarly, the DP of the A-PCL was calculated from the integration ratio of the signals at $\delta = 4.0$ ($-CH_2O-$ of the backbones of PCL) and $\delta = 2.0$ ppm (alkyne group) (Figure 1b). The controlled nature of the polymerizations could be demonstrated by the fact that the PCL samples showed a narrow PDI of 1.09–1.13 and the DP obtained was in agreement with the feed ratio of monomer to initiator and the monomer conversion. The molecular weight of the PCL is controlled to be relatively low with a consideration that the polymer will show amphiphilic properties after the following end-modification with saccharides.

Figure 2. ^1H NMR spectra of Mal-PCL, by Michael addition (a) and click chemistry (b) (solvent DMSO-d_6).

Synthesis of Mal-PCL by Michael Addition

Michael addition of maleimide and amino group is used widely for the modification of polymers. The addition reaction proceeded smoothly in polar solvents. Mal-NH_2 and M-PCL were allowed to react in DMSO at room temperature (Scheme 2) to afford Mal-PCL with different DPs (10, 17 and 22). The feed ratio of Mal-NH_2 to M-PCL was 1.5:1, and the excess Mal-NH_2 was removed by precipitation in methanol. Figure 2a shows the ^1H NMR spectrum of the addition product between Mal-NH_2 and M-PCL$_{17}$. It can be seen that successful conjugation of Mal-NH_2 and M-PCL$_{17}$ was verified by the complete disappearance of the maleimido signals ($\delta = 6.8$ ppm) and the appearance of the resonance of saccharide ($\delta = 5.0$ ppm). Moreover, the integral ratio of the signals at $\delta = 5.0$ ($H_{1'}$ in maltose) and $\delta = 4.0$ ppm ($-CH_2OC(O)CH_2-$) was 1:34 (Figure 2 Figure 2a). The products showed an increase of $\overline{M}_n$ and a slight increase of PDI compared with M-PCL (Table 2). These results indicate that the amino groups reacted selectively with the maleimido-end groups of PCL and the addition reaction conditions did not induce the degradation of PCL. The molecular weight distributions slightly increase after

the Michel reaction, which suggests a slight degradation during the reaction as confirmed by control experiments.

Synthesis of Saccharide-Terminated PCL by Click Chemistry

In a click reaction, the solubility of the Cu^I catalyst in solvent is important,[13b] so different conditions (solvents, ligands) were examined. Acetone and PMDETA were found to be suitable for the present reactions. Mal-PCL could be obtained by the click reaction between Mal-N_3 and A-PCL in acetone with Cu^I as catalyst. Although the azido-containing saccharide had a low solubility in acetone, the

Table 2. Michael addition of Mal-PCL and Mal-NH_2.

Sample[a]	DP[b]	$\overline{M}_n$[c]	$\overline{M}_w/\overline{M}_n$[c]	Yield[d]
				%
Mal-PCL$_{10}$	10	4 100	1.38	82
Mal-PCL$_{17}$	17	4 800	1.21	57
Mal-PCL$_{22}$	22	5 300	1.19	84

[a]Carried out in DMSO at r. t. for 48 h. Feed ratio of Mal-NH_2 to M-PCL was 1.5:1; [b]Degree of polymerization calculated from ^1H NMR spectroscopy; [c]Determined by GPC; [d]Determined gravimetrically.

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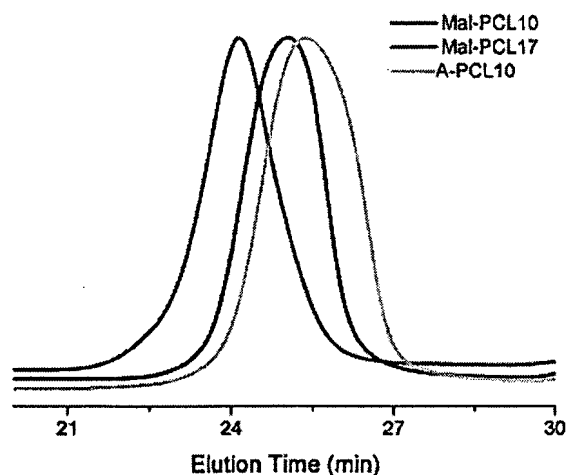
Figure 3. GPC traces of A-PCL, Mal-PCL$_{10}$, and Mal-PCL$_{17}$ obtained by click chemistry.

Table 3. Synthesis of saccharide-containing PCL by click chemistry.

Sample[a]	A-PCL	$\overline{M}_n$[c]	PDI[c]	Yield[d] %
Glu-PCL$_{17}$[b]	A-PCL$_{17}$	4 200	1.03	67
Mal-PCL$_{10}$	A-PCL$_{10}$	3 100	1.11	80
Mal-PCL$_{17}$	A-PCL$_{17}$	4 400	1.14	57
TEMal-PCL$_{10}$	A-PCL$_{10}$	3 500	1.10	60
TEMal-PCL$_{17}$	A-PCL$_{17}$	4 700	1.13	65
TEMal-PCL$_{35}$	A-PCL$_{35}$	8 500	1.14	85

[a][Cu^{I}Br]:[PMDETA]:[A-PCL]:[saccharide-N$_3$] = 2:2:1:1.5; concentration of the polyesters is 100 mg · mL^{-1}, and the reaction was carried out at 35 °C for 48 h; [b]Carried out between Glu-N$_3$ and A-PCL$_{17}$, [Cu^{I}Br]:[PMDETA]:[A-PCL]:[Glu-N$_3$] = 2:2:1:2; [c]Determined by GPC; [d]Determined gravimetrically.

click reaction could be accomplished under this mild condition because of high reactivity of this method. From the ^{1}H NMR spectrum of Mal-PCL$_{17}$ (Figure 2b), signals at 7.9 ppm indicate the formation of triazole, and conversion of the end group of A-PCL$_{17}$ could be calculated from the integral ratio of the signals at 7.9 and 4.0 ppm, which confirmed complete transition of alkyne group to triazole. GPC traces of Mal-PCL obtained by click chemistry indicated a narrow PDI of the modified PCL (Figure 3). The click reaction between Mal-N$_3$ and A-PCL did not cause any degradation of PCL. The data of for the click reactions are listed in Table 3. The modified PCL is slightly soluble in methanol and H$_2$O, as such the yields were relatively low.

Similarly, Glu-PCL and TEMal-PCL could also be prepared by the same procedures (Table 3). Their structures were confirmed by NMR spectroscopy (Figure 4), and these polymers also showed narrow molecular weight distributions (Figure 5).

To confirm the structure of Mal-PCL, Mal-PCL was synthesized in another way. That is, BnMal-PCL was used to conjugate to the end of A-PCL followed by deprotection of the benzyl group with Pd/C/H$_2$. Click chemistry was carried out under the same conditions as stated above. BnMal-PCL with a narrow PDI of 1.11–1.29 was obtained in good yield. The ^{1}H NMR spectrum of BnMal-PCL$_{17}$ is shown in Figure 6a, and the signals at $\delta = 7.75$ (the triazole) and 7.2 ppm (PhCH$_2$O–) confirm the formation of BnMal-PCL. Deprotection of the benzyl

groups was carried out with Pd/C as catalyst in EtOAc/CH$_3$OH (2:1, v/v) at room temperature for 72 h. Disappearance of the signals at $\delta = 7.2$ ppm (Ph-) and the shift of the signals from $\delta = 7.75$ to 7.9 ppm (the triazole) indicate that the deprotection was successful (Figure 6b). The maltose content in Mal-PCL could be calculated by the integral ratio of the signals at $\delta = 4.1$ (–C(O)OCH$_2$–) and 5.1 ppm (1'-protons in maltose), and agreed well with that before deprotection. This spectrum was essentially the same as the ^{1}H NMR spectrum of Mal-PCL synthesized directly from Mal-N$_3$ and A-PCL (Figure 2b), and confirmed the desired structure of Mal-PCL.

For more analytical details please see Supporting Information.
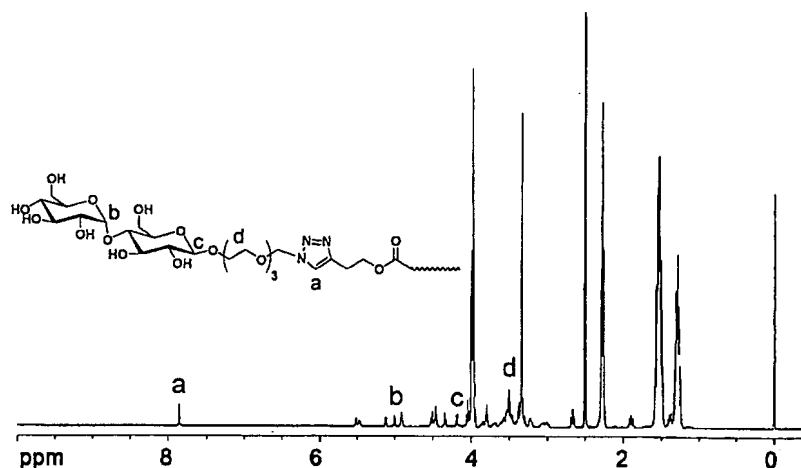
Figure 4. ^{1}H NMR spectrum of TEMal-PCL$_{17}$ in DMSO-d_6.
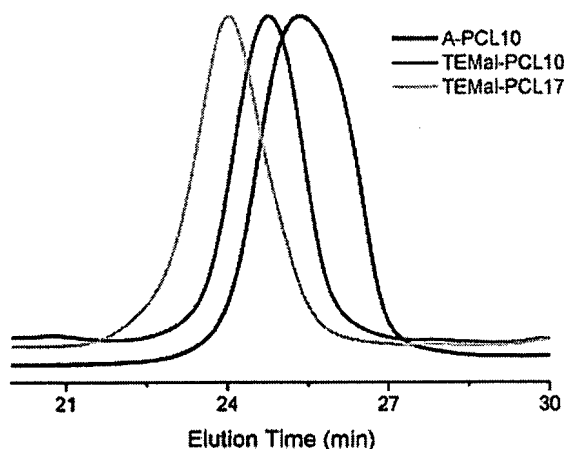
Figure 5. GPC traces of A-PCL$_{10}$, TEMal-PCL$_{10}$, and TEMal-PCL$_{17}$.

Aggregation of the Amphiphilic Polymer

PCL with a hydrophilic segment can aggregate in water to form polymer micelles or vesicles.[24] The aggregation behavior of the above prepared saccharide-terminated PCL has been examined in water. For Glu-PCL$_{17}$ and Mal-PCL, the hydrophilicity is too low for the polymer to form stable aggregates in aqueous solution. However, TEMal-PCL$_{10}$ and TEMal-PCL$_{17}$ were found to form stable aggregates in water with diameters of about 220 and 210 nm, as determined by DLS (Figure 7). The aggregate morphology was examined by TEM (Figure 8). It is seen that aggregates formed by TEMal-PCL$_{10}$ and TEMal-PCL$_{17}$ were vesicle-like

in structure, with average external diameters of 80 and 150 nm, respectively. The shape of the TEMal-PCL$_{10}$ aggregates was irregular while that of TEMal-PCL$_{17}$ was mainly spherical. The diameters determined from the DLS measurements were larger than those from TEM, which might be attributed to the shrinkage of aggregates during preparation and observation with TEM. Both the morphologies and the sizes of the aggregates do not change within one week.

From the nature of the polymers, it can be deduced that the surface of the aggregates is covered with saccharides. In order to confirm this, interaction of the aggregates with Con A, a member of the lectin family that selectively binds α-D-mannose and α-D-glucose, was investigated by measuring the transmittance of the mixtures at 500 nm.[16,25] The result is shown in Figure 9. It can be clearly seen that the transmittance of the mixture of TEMal-PCL$_{10}$ aggregates and Con A quickly decreases, which is a result of the specific interaction between Con A and the maltose moiety on the surface of the aggregates.

Conclusion

New saccharide-terminated PCL has been successfully prepared by Michael addition and click chemistry. Both methods represent a convenient synthesis of end-modified aliphatic polyesters without degradation of polyester and other side reactions. These amphiphilic polyesters can form vesicle-like aggregates in water. With saccharide on
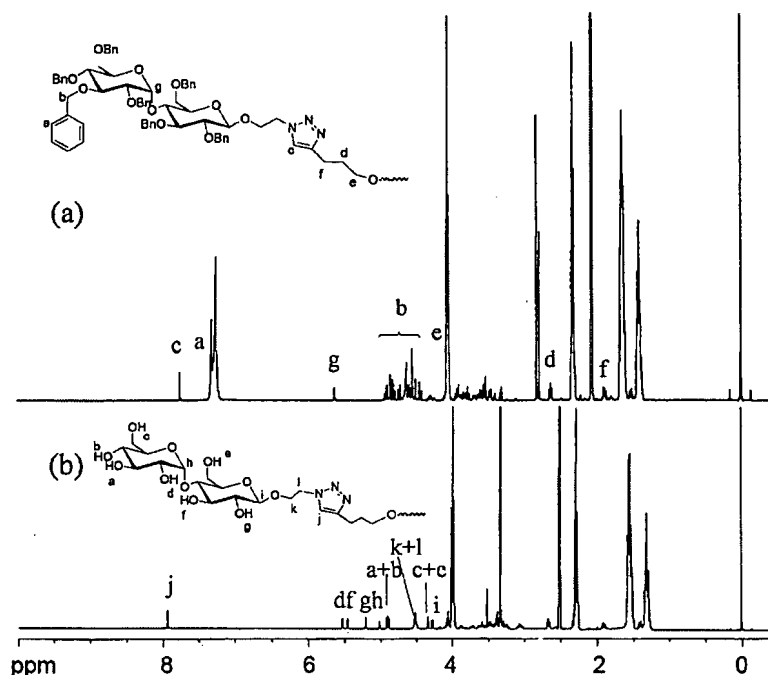
Figure 6. ^{1}H NMR spectra of BnMal-PCL$_{17}$ in acetone-d_6 (a) and Mal-PCL$_{17}$ in DMSO-d_6 (b).

Synthesis of Saccharide-Terminated Poly(ε-caprolactone) via Michael Addition...

Macromolecular
Chemistry and Physics

a)

Rh (nm)

b)

Rh (nm)
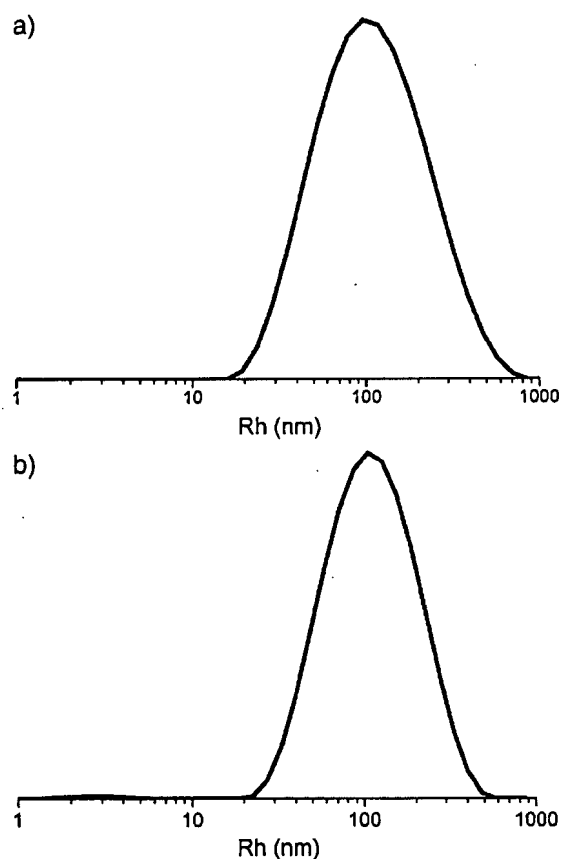
Figure 7. DLS traces of the aggregates formed by 0.2 mg · mL^{-1} of TEMal-PCL$_{10}$ (a) and 0.1 mg · mL^{-1} of TEMal-PCL$_{17}$ (b).

a)

300 nm

b)

300 nm
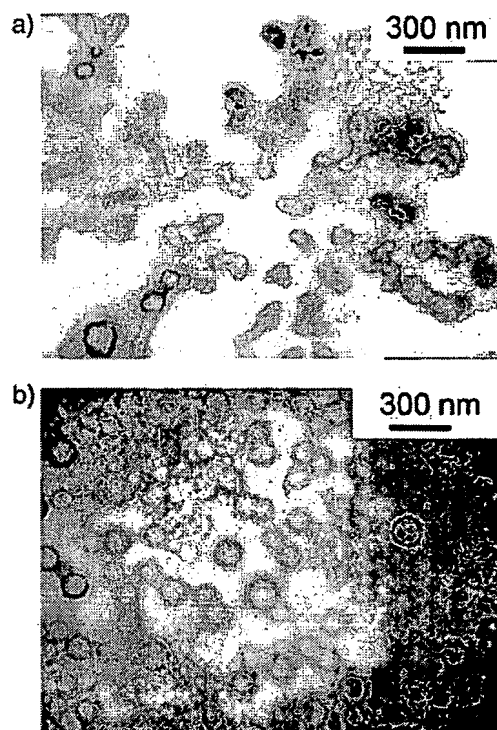
Figure 8. TEM images of aggregates formed by TEMal-PCL$_{10}$ (a) and TEMal-PCL$_{17}$ (b) at a concentration of 0.5 mg · mL^{-1}.
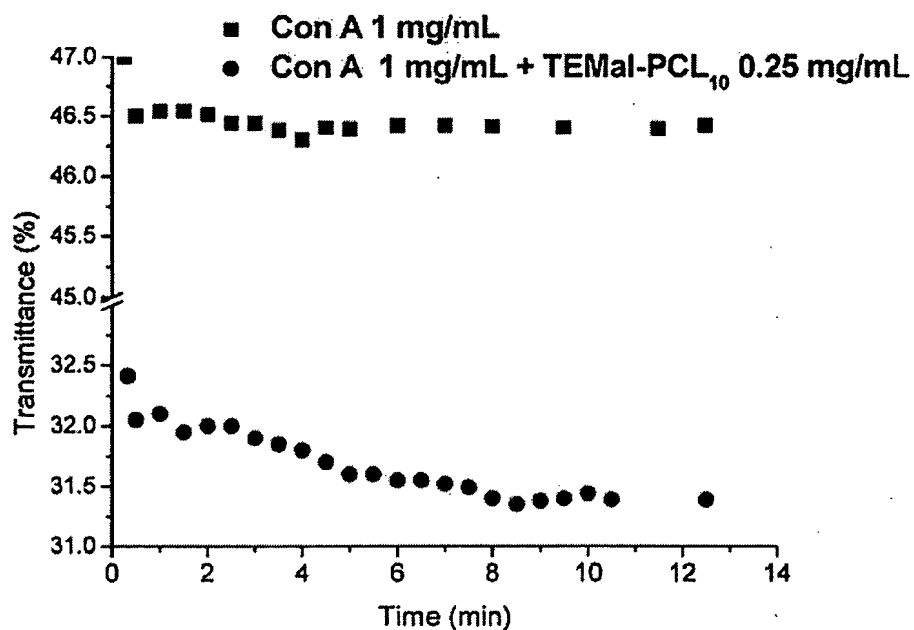
Figure 9. The interaction of aggregates formed by TEMal-PCL$_{10}$ and Con A (λ = 500 nm).

the surface, these aggregates may be useful for future study as targeting drug-carrier systems.

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Click Chemistry in Materials Synthesis. II. Acid-Swellable Crosslinked Polymers Made by Copper-Catalyzed Azide–Alkyne Cycloaddition

CHUNMEI LI, M. G. FINN

Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

ABSTRACT: A highly crosslinked hyperbranched polymer that rapidly swells and shrinks in a halogenated solvent in response to the addition of an acid or base has been prepared by Cu(I) catalysis of the reaction between a diazide and an amine-containing trialkyne. The triazole linkages in the polymer are highly stable and may also play a role in the swelling behavior. The swelling–deswelling process is reversible. © 2006 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 44: 5513–5518, 2006

Keywords: alkynes; azides; click chemistry; cycloaddition; heteroatom-containing polymers; hyperbranched polymers; nonlinear polymers; stimuli-responsive; swelling

INTRODUCTION

Polymers that exhibit reversible swelling behaviors in response to environmental stimuli can be used to design smart materials. Although hydrogels that respond to the temperature, pH, and other factors have been extensively studied for this purpose,[1–10] materials displaying similar properties in organic solvents have received comparatively little attention. Here we describe a highly crosslinked hyperbranched polymer that rapidly swells and shrinks in a halogenated solvent in response to the addition of an acid or base. It was discovered in the early stages of our efforts to apply the copper(I)-catalyzed azide–alkyne cycloaddition click reaction[11,12] to materials synthesis.[13] The process is rapid and tolerant of a broad range of solvents and functional groups, making it of interest in the assembly of building blocks for organic, biological, and materials purposes.[14] One report of hyperbranched materials made in this manner has previously appeared.[15]

EXPERIMENTAL

Synthesis of 1,6-Diazidohexane (1)

A two-phase mixture of 1,6-diiodohexane (6.76 g, 20 mmol) and methanol (MeOH, 30 mL) was treated with a solution of NaN_3 (3.64 g, 56 mmol) in 10 mL of water. The mixture was heated at reflux overnight. The product formed as an oil on top of the aqueous MeOH layer. The mixture was cooled to room temperature, and the two layers separated. The oil was added to a solution of $AgNO_3$ in MeOH, and the mixture was filtered. The filtrate was concentrated, dissolved in dichloromethane, washed with water, and dried over $MgSO_4$. The evaporation of the solvent immediately before use afforded 1 in a 65% yield (Caution: although we have not had any prob-

lems with the material, any small-molecule azide should be handled carefully, should not be distilled, and should be stored in a solution rather than in its pure form).

Polymer Synthesis

The parent material **3** was prepared by the dissolution of **1** (0.84 g, 5 mmol) and tripropargylamine (**2**; 3.33 mmol) in *t*-BuOH (5 mL). Water (5 mL) and hexanes (1 mL) were then added. A freshly prepared solution of sodium ascorbate (100 mg) and $CuSO_4 \cdot 5H_2O$ (62.5 mg, 0.25 mmol) in a minimum amount of water was then added to the monomer solution on an orbital shaker. The polymer product started precipitating within 1 min, and the reaction was allowed to proceed overnight. The liquid was decanted, and the remaining solid was washed with 0.1 M disodium ethylenediaminetetraacetic acid (Na_2EDTA) aqueous disodium tetraacetic acid at 90 °C until the washings were colorless (three to five times). The polymer was washed with water and then acetone and was dried under reduced pressure. Materials incorporating both **2** and 1,7-octadiyne (**4**) were made with the same procedure with the appropriate monomer mixtures. All polymers were isolated in 80–90% yields.

Polymer Characterization

Thermogravimetric analysis (TGA) was performed with a TA Instruments Universal V3.2B instrument. IR spectroscopy was performed on KBr pellets with a MIDAC EM200 instrument. Two-photon fluorescence microscopy (excitation at 580 nm) was performed at the Combinatorial Materials Research Laboratory at BASF Ludwigshafen on an instrument constructed from commercial parts (a Leica microscope and a Coherent laser). We were unable to employ gel permeation chromatography or any other solution-phase technique because of the insoluble nature of the crosslinked polymers. Similarly, these materials were resistant to combustion analysis, producing variable amounts of ash.

Swelling Measurements

The swelling behaviors of the polymers were studied gravimetrically. The polymers were weighed and placed in a 1:3 (v/v) CF_3CO_2H/solvent mixture (solvent = CH_2Cl_2 or $C_6H_5CF_3$) at room temperature. The samples were periodically

removed from the solvent, and excess solvent was carried off by brief contact with filter paper. The weight was then recorded immediately, and the sample was returned to the same acid solution. This procedure was continued until a constant weight was attained. The values shown in Figure 1 are the averages of three independent measurements for each sample.

To measure the reversible swelling and deswelling, the initially dry polymer was immersed in a 1:3 (v/v) CF_3CO_2H/solvent mixture and allowed to attain equilibrium swelling as previously discussed. The excess solution was poured off, and the gel was washed extensively with the solvent. A 1:3 (v/v) Et_3N/solvent mixture was then added, and the mixture was allowed to stand for 1 min. The polymer powder was recovered by filtration and weighed immediately. This swelling/deswelling process was repeated as discussed previously.

RESULTS AND DISCUSSION

Crosslinked polymer **3** was conveniently synthesized from A_2 and B_3 components **1** and **2**, as shown in Figure 1. The [3+2] cycloaddition of these units provides 1,2,3-triazoles, which are extraordinarily stable to other chemical reagents, high temperatures, oxidation, and reduction. The reaction is a fusion process leaving no byproducts, and the copper catalysis is rapid and is often stimulated by polydentate triazoles.[16–18] This chemistry may therefore be expected to provide efficient polymer formation and the possibility of highly active catalytic domains in the developing polymer to enhance crosslinking. **2** was selected as the B_3 component in part because tris(triazolylamine)s derived from this building block are among the most effective ligands in promoting the cycloaddition process in general.[16,17]

The polymerization reactions were carried out in 1:1 H_2O/*t*-BuOH, with hexanes added as a porogen and no precautions to exclude air. Catalysis was provided by Cu(I) generated *in situ* from 5 mol % $CuSO_4 \cdot 5H_2O$ and 10 mol % sodium ascorbate. The precipitated polymer formed within a minute of the addition of the catalyst to the monomer mixtures at room temperature, although the temperature of the solution did not rise by more than a few degrees during this initial rapid cycloaddition phase. After reaction times of 12–16 h, the products were isolated by filtration, and the copper trapped in the polymer
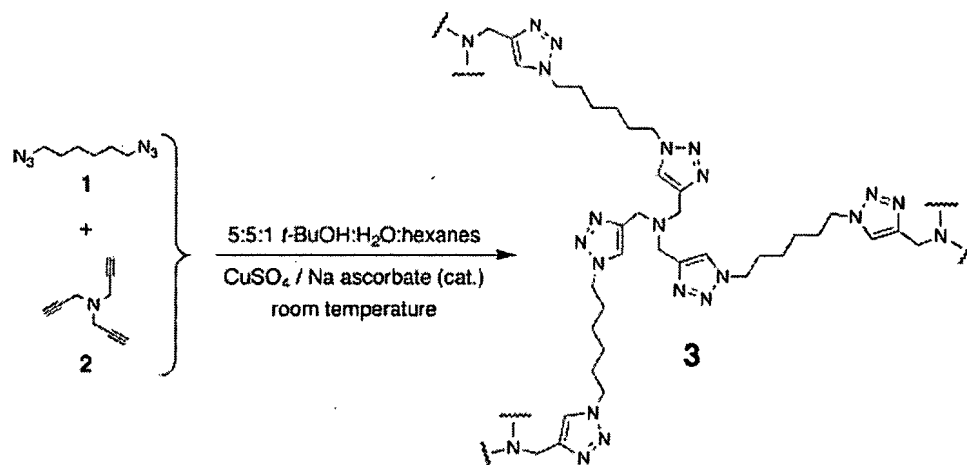
Figure 1. Synthesis of hyperbranched polymer 3.

was extracted by repeated treatment with hot aqueous ethylenediaminetetraacetic acid. The resulting materials were isolated in excellent yields (>95%) and were insoluble in every solvent tested [CH_2Cl_2, MeOH, CH_3CN, tetrahydrofuran, hexanes, EtOAc, dimethyl sulfoxide, dimethylformamide, dimethylacetamide, Et_2O, N-methylpyrrolidone, trifluoroacetic acid (TFA), and pyridine]; this was consistent with the expected high degree of crosslinking from A_2+B_3 reactions.

The presence of unreacted azide and alkyne groups in **3** was revealed by the presence of a small band at 2102 cm^{-1} in the IR spectrum. However, a colorimetric test for azide, which required the reduction of the azide group by added phosphine,[19] was negative in all cases. This suggests that the unreacted azide groups (and therefore the alkynes as well) are likely in hindered regions of the polymer network that are largely inaccessible to solution-phase species. The existence of such tightly packed domains is expected in many crosslinked materials.[20]

Polymer **3** was obtained as a brown solid inert to CH_2Cl_2 and all other solvents. The suspension of the material in 25% TFA in CH_2Cl_2 caused the polymer, which was initially less dense than the solvent mixture, to absorb the solvent and sink to the bottom of the vial in a few minutes. Within the first 10 min, the material absorbed 18 times its weight in solvent and then underwent a slower absorption phase until saturation was attained. During this process, the polymer expanded by approximately fivefold in volume and assumed the appearance of a gel rather than a powder. The solvent contents of the gel (by weight) as a function of time are shown in Figure 2. Simi-

lar behavior was observed in a less volatile mixture of 1:3 TFA/α,α,α-trifluorotoluene (Fig. 2). The level of solvent absorption observed for **3** is roughly in the middle of the range of figures reported in the literature for other polymers.[21–24]

Polymer **3** did not swell in the presence of acid under aqueous conditions or in any other organic solvent. When a few drops of water were added to the expanded gel after the TFA treatment, the polymer precipitated immediately. The swelling process did not occur when trichloroacetic acid or anhydrous HCl was used in place of TFA under otherwise identical conditions. Furthermore, the
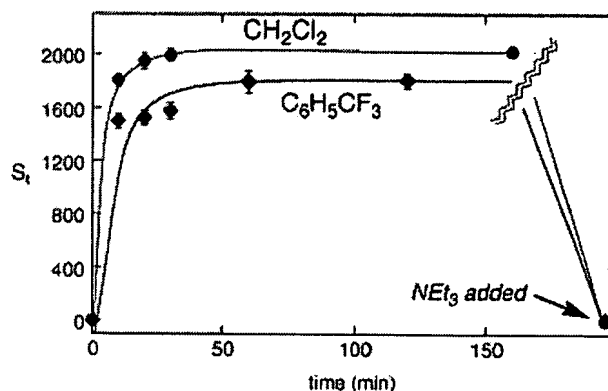
Figure 2. Swelling of polymer **3** as a function of time after the addition of TFA as a 25% solution in the indicated solvent [S_t = 100 × $(W_t - W_0)/W_0$, where S_t is the swelling at time t, W_t is the weight of the gel at time t, and W_0 is the dry weight of the polymer before swelling]. The treatment of the swollen material with triethylamine was performed at an arbitrary time after equilibrium swelling had been attained.
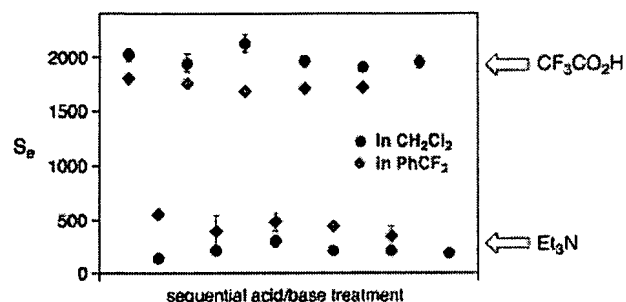
Figure 3. Reversibility of the swelling of polymer **3** by a sequential treatment with an acid and a base in the indicated solvent. The equilibrium swelling (S_e) is equal to the swelling at time t after saturation has been achieved (see Fig. 2).

pretreatment of the polymer with Cl_3CCO_2H blocked the TFA-induced swelling behavior. These observations suggest that a very strong acid is required for swelling to occur and that the trifluoroacetate counterion may play a role (vs chloride from HCl). Apparently, the protonation

of only the tertiary amine groups, which would be expected with all of the acids tested, does not induce sufficient charge–charge repulsion to influence the polymer morphology. Because the acidities of TFA and the conjugate acid of 1,4-triazoles are roughly equivalent (both ca. 0 in water),[25] a substantial degree of protonation of the triazole linkages in the polymer may be expected in a TFA solution.

After the acidified swollen polymer was washed, a treatment with triethylamine caused **3** to lose almost all of its accumulated trapped solvent mass. Many cycles of acid/base treatment were performed with little or no loss in the measured retention of the solvent (Fig. 3). However, although solvent retention was reversible, the physical expansion of the material was not: after the initial triggering with acid, the material lost only a fraction of its expanded volume upon its treatment with a base. Two-photon scanning microscopy images of the polymer before swelling and after deswelling (Fig. 4) confirmed that the
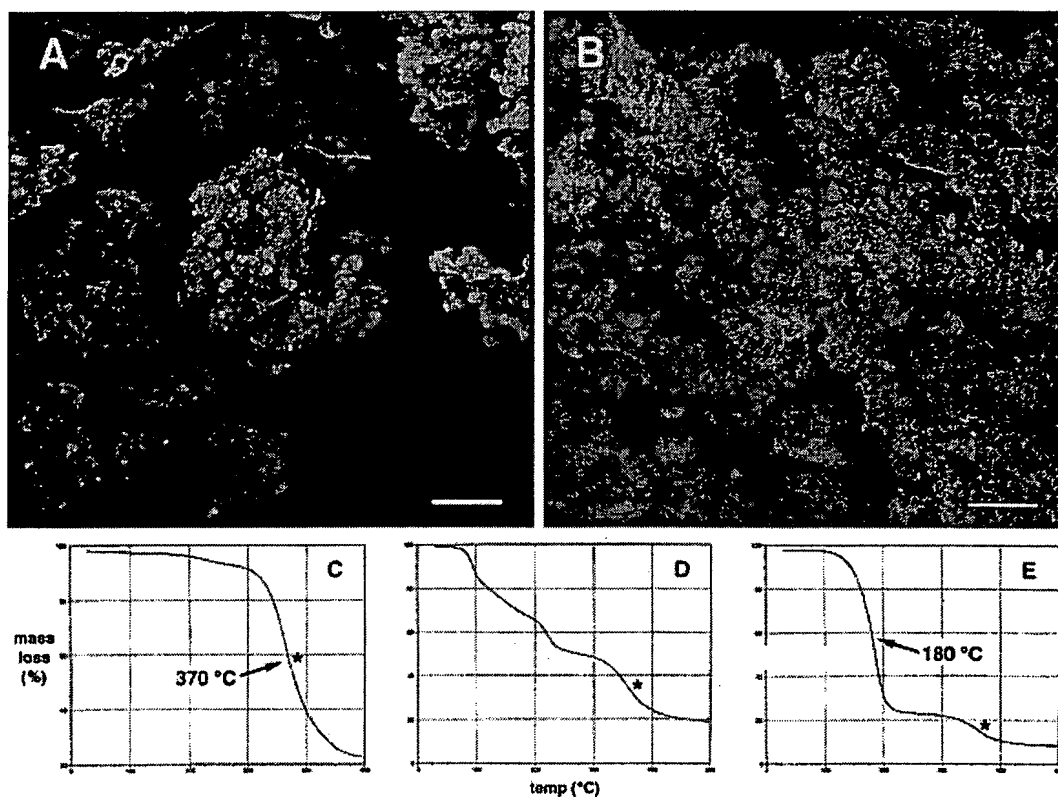
Figure 4. Characterization of hyperbranched material **3**: (A) two-photon microscopy before swelling (scale bar = 50 μm), (B) two-photon microscopy after swelling and deswelling by a sequential treatment with CF_3CO_2H and Et_3N in CH_2Cl_2 (scale bar = 50 μm), (C) TGA of **3** before swelling, (D) TGA of **3** swollen with CF_3CO_2H in CH_2Cl_2, and (E) TGA of **3** after deswelling with Et_3N. The asterisk marks the high-temperature transition (ca. 370 °C) in each sample.
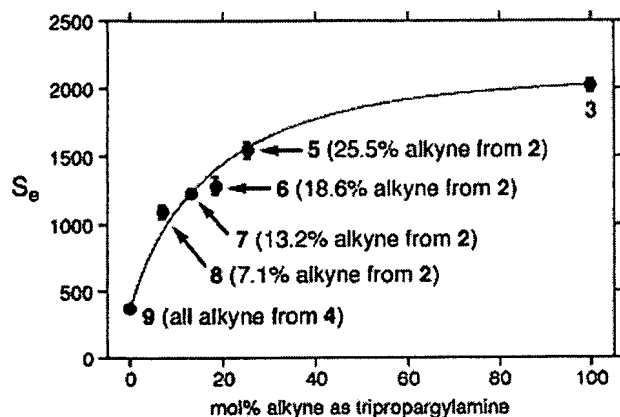
Figure 5. Swelling of polymers (in 1:3 CF_3CO_2H/CH_2Cl_2) containing various ratios of 2 and 4 (S_e is the equilibrium swelling).

originally polymer was densely packed and expanded after protonation. TGA of polymer **3** showed that it experienced a single major transition at approximately 370 °C [Fig. 4(C)], presumably representing decomposition of the highly stable network. The swelled [Fig. 4(D)] and deswelled [Fig. 4(E)] materials retained this transition, along with major losses of mass proposed to correspond with the ejection of the solvent and TFA or triethylammonium trifluoroacetate, respectively. Tris(benzyltriazolylmethyl)-amine, the product of the reaction of **2** and benzyl azide,[16] withstands standing for extended periods in 50% TFA in $CDCl_3$ with no decomposition, as determined by NMR. Because this benzyl derivative would be expected to be more acid-sensitive than the crosslinking triazole moieties of **3**, it is unlikely that the chemical structure of **3** is irreversibly changed by protonation.

To study the influence of the composition of the material on its swelling behavior, a series of polymers was made with an equimolar ratio of azide and alkyne groups, as used previously, but with alkynes provided by differing amounts of **2** and **4**. A direct relationship between the swelling ability and the amount of tripodal amine in the monomer mixture feedstock was observed (Fig. 5), suggesting that the more highly crosslinked and densely functionalized the material is, the more it swells upon acidification. Two additional points are notable. First, the relative rates of monomer incorporation were not measured, but it is a good assumption that the cycloaddition rates of the first alkyne unit of **2** and **4** are roughly equivalent. However, the second and/or third alkynes of **2** may undergo much more rapid cycloaddition

than the first because of a favorable conformation, which presents these alkynes to a tethered active copper catalyst.[26] Second, linear polymer **9**, made from **4** and **1** and therefore lacking tertiary amine groups, still swells to a measurable degree upon its treatment with CF_3CO_2H.

CONCLUSIONS

We have shown here that copper-catalyzed azide–alkyne cycloaddition is able to assemble highly stable and crosslinked materials under mild conditions in aqueous/organic solvent mixtures. The inclusion of a tertiary amine functionality in the polymer enhances a mechanical response of the material to TFA, inducing marked swelling and solvent retention. The excellent functional group and solvent tolerance of the azide–alkyne cycloaddition reaction has thus far been exploited in materials synthesis mainly to decorate preformed polymers or join macromolecular pieces.[14] 1,2,3-Triazoles are noteworthy for their pseudoaromatic nature (providing the ability to engage in π-stacking), large dipole moment,[25] and excellent hydrogen-bonding ability. Perhaps because of these types of molecular interactions, which may be expected to induce phase separation, we have found that main-chain 1,2,3-triazole polymers are often highly insoluble. We believe that this property and the great stability of triazoles can be used to advantage in certain applications,[13,15] such as the construction of novel colloidal and porous materials, solid supports for chemical synthesis and sensing, and chromatographic stationary phases.[27] The synthesis and properties of such materials are the subjects of current investigation in our laboratory.

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Click Assembly of 1,2,3-Triazole-Linked Dendrimers, Including Ferrocenyl Dendrimers, Which Sense Both Oxo Anions and Metal Cations**

*Cátia Ornelas, Jaime Ruiz Aranzaes, Eric Cloutet, Sandra Alves, and Didier Astruc**
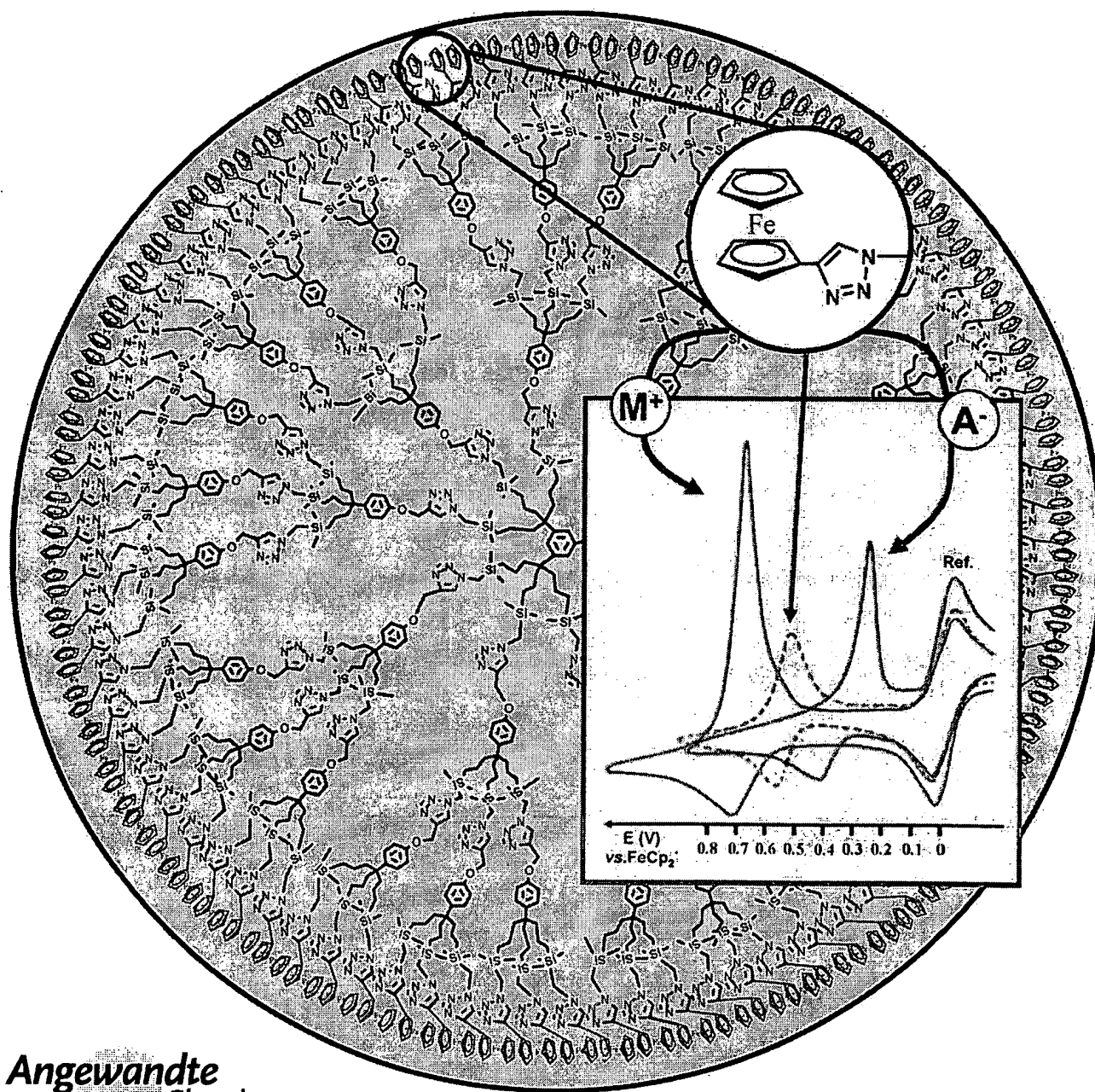
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Dendrimers are well-defined macromolecules[1] whose multi-faceted supramolecular properties can be applied to various fields of nanoscience, such as vectors, sensors, and green catalysts.[1,2] We have now assembled dendrimers linked by 1,2,3-triazole heterocycles by the Huisgen 1,3-dipolar cyclo-addition between azides and alkynes, a reaction that has recently been greatly improved and defined by Sharpless as "click chemistry" because of its regioselectivity and catalytic course in the presence of Cu^I, its tolerance of a wide range of functionalities, and its high yields in water.[3] Recently there have been a few reports of the linkage of dendrons to cores using click reactions.[4] Our goal was threefold: 1) to investigate the full click assembly of 9-, 27-, and 81-tethered dendrimers; 2) to functionalize these dendrimers with a ferrocenyl group also by click chemistry; and 3) to investigate the ability of the 1,2,3-triazole rings located inside these metallodendrimers to recognize, bind, and sense oxo anions and metal cations using the ferrocenyl termini as a redox monitor[2b] directly attached to the triazole ring.

For the construction of the dendrimers (Scheme 1) we used the $1 \rightarrow 3$ C connectivity pioneered by Newkome.[5] The known nona-allylation of [FeCp(η^6-mesitylene)](PF$_6$) (1; Cp = C$_5$H$_5$) quantitatively yielded the nona-allyl dendritic core 2 on a large scale after visible-light photolysis to remove the metal moiety.[6] Likewise, the known triallyl–phenol dendronic brick para-HOC$_6$H$_4${C(CH$_2$CH=CH$_2$)$_3$}, obtained from the one-pot reaction of [FeCp(η^6-para-ethoxytoluene)](PF$_6$) with allyl bromide and tBuOK,[6] was synthesized to serve as the precursor of the building block used for dendritic progression.

Hydrosilylation of the terminal olefinic bonds of 2, a reaction pioneered in dendrimer synthesis by van Leeuwen et al.,[7] was carried out with HSiMe$_2$(CH$_2$Cl), the Karsted catalyst, to give the nona-chloromethyl(dimethyl)silyl inter-mediate regioselectively, which, upon treatment with NaN$_3$, provided the nona-azide 4. Functionalization of the triallyl–phenol dendron with propargyl bromide at the phenol focal point gave dendron 5, which is suitable for click chemistry. The Cu^I-induced click reaction between 4 and 5 in water/THF

yielded the first-generation (G$_1$) 27-allyl dendrimer 6, which contains nine 1,2,3-triazole links and was characterized by its molecular peak at 3937.42 [M + Na$^+$] in the MALDI-TOF mass spectrum (calcd for C$_{234}$H$_{327}$N$_{27}$O$_9$Si$_9$Na: 3938.04). Repetition of this sequence of reactions yielded the 27-azido intermediate 8 and subsequently the 81-allyl second-generation (G$_2$) dendrimer 9, which contains 36 triazole links in two layers (9 + 27).

Note that whereas this click reaction is usually catalytic with Cu^I (5% Cu^I is used by most authors), the present click dendrimer synthesis requires a stoichiometric amount of Cu^I because the metal remains trapped inside the dendrimer and is only removed as [Cu(NH$_3$)$_6$]$^+$ ions by washing with aqueous ammonia solution. This feature was further confirmed by recognition and titration studies of the click dendrimers with Cu^I (see below). On the other hand, an advantage of this procedural variation is that our click reaction is much faster than the standard procedure (0.5 h at 20°C instead of 16 h).

To monitor the functions of the triazole groups, such as molecular recognition or metal complexation, by cyclic voltammetry (CV), we attached ferrocenyl groups to the triazole units at the periphery of the dendrimers by treating the polyazido dendrimers 4, 8, and 11 (generations G$_0$, G$_1$, and G$_2$, respectively) with ethynylferrocene (FcC≡CH) to yield the poly-1,2,3-triazolylferrocenyl dendrimers 12, 13, and 14, respectively, under ambient conditions in water/THF [Eq. (1)–(3)].

Dendr-(CH$_2$N$_3$)$_9$ (4) + FcC≡CH → Dendr-(CH$_2$–N$_3$C$_2$H–Fc)$_9$ (12)

(1)

Dendr-(CH$_2$N$_3$)$_{27}$ (8) + FcC≡CH → Dendr-(CH$_2$–N$_3$C$_2$H–Fc)$_{27}$ (13)

(2)

Dendr-(CH$_2$N$_3$)$_{81}$ (11) + FcC≡CH → Dendr-(CH$_2$–N$_3$C$_2$H–Fc)$_{81}$ (14)

(3)
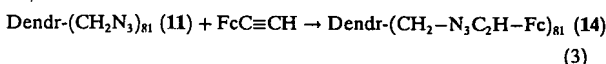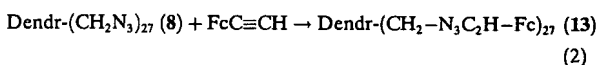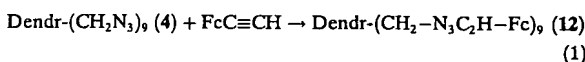
These poly-1,2,3-triazolylferrocenyl dendrimers were characterized by ^{1}H, ^{13}C, 2D ^{1}H-^{13}C correlation, and ^{29}Si NMR spectroscopy, MALDI-TOF spectrometry (G$_0$, 12), and size-exclusion chromatography (SEC). SEC (see Supporting Information, page 36) shows the size progression from G$_0$ (12) to G$_2$ (14) and the low polydispersity (1.00 to 1.02). The G$_2$ dendrimer 14 was also characterized by dynamic light scattering (DLS), its hydrodynamic diameter in dichloro-methane solution being (12 ± 0.5) nm.

The ferrocenyl dendrimers 12–14 show a single, fully reversible CV wave for all the equivalent (but distant) ferrocenyl groups. The potentials of these groups are similar, the electrostatic factor apparently being very weak.[2b,8] Determination of the number of electrons included in this wave using the Bard–Anson equation[9] with decamethylfer-rocene as the internal reference gives 9 ± 1, 29 ± 3, and 87 ± 9 electrons for 12, 13, and 14 respectively, in good agreement with the theoretical numbers. The very slight excess found is probably due to a slight adsorption phenomenon, which increases as the dendrimer generation increases.

Recognition of oxo anions by ferrocenyl-based redox sensors has been studied thoroughly using various endo

[*] C. Ornelas, Dr. J. Ruiz Aranzaes, Prof. D. Astruc
Nanosciences Moléculaires et Catalyse
LCOO, UMR CNRS no. 5802
Université Bordeaux I
33405 Talence Cedex (France)
Fax: (+33) 5-4000-6646
E-mail: d.astruc@lcoo.u-bordeaux1.fr
Homepage: http://costd12.workshop.free.fr/Fer/

Dr. S. Alves
LCSOB, UMR CNRS N° 7613
Université Paris VI
75252 Paris Cedex (France)

Dr. E. Cloutet
LCPO, UMR CNRS N° 5629
Université Bordeaux I
33405 Talence Cedex (France)

Supporting Information for this article is available on the WWW under http://www.angewandte.org or from the author.

a) H$_2$C=CHCH$_2$Br
KOH, THF, RT,
3 days

b) $h\nu_{vis}$, MeCN

a) HSi(CH$_3$)$_2$(CH$_2$Cl),
Karsted catalyst
ether, RT, 13 h

b) NaN$_3$, DMF
16 h, 80°C

1

FE = [η^5-CpFe]$^+$

2

4

CuSO$_4$
sodium ascorbate
THF, H$_2$O

5

RT, 30 min

a) HSi(CH$_3$)$_2$(CH$_2$Cl),
Karsted catalyst
ether, RT, 13 h

b) NaN$_3$, DMF
16 h, 80°C

8

6

CuSO$_4$
sodium ascorbate
THF, H$_2$O

RT, 30 min

9
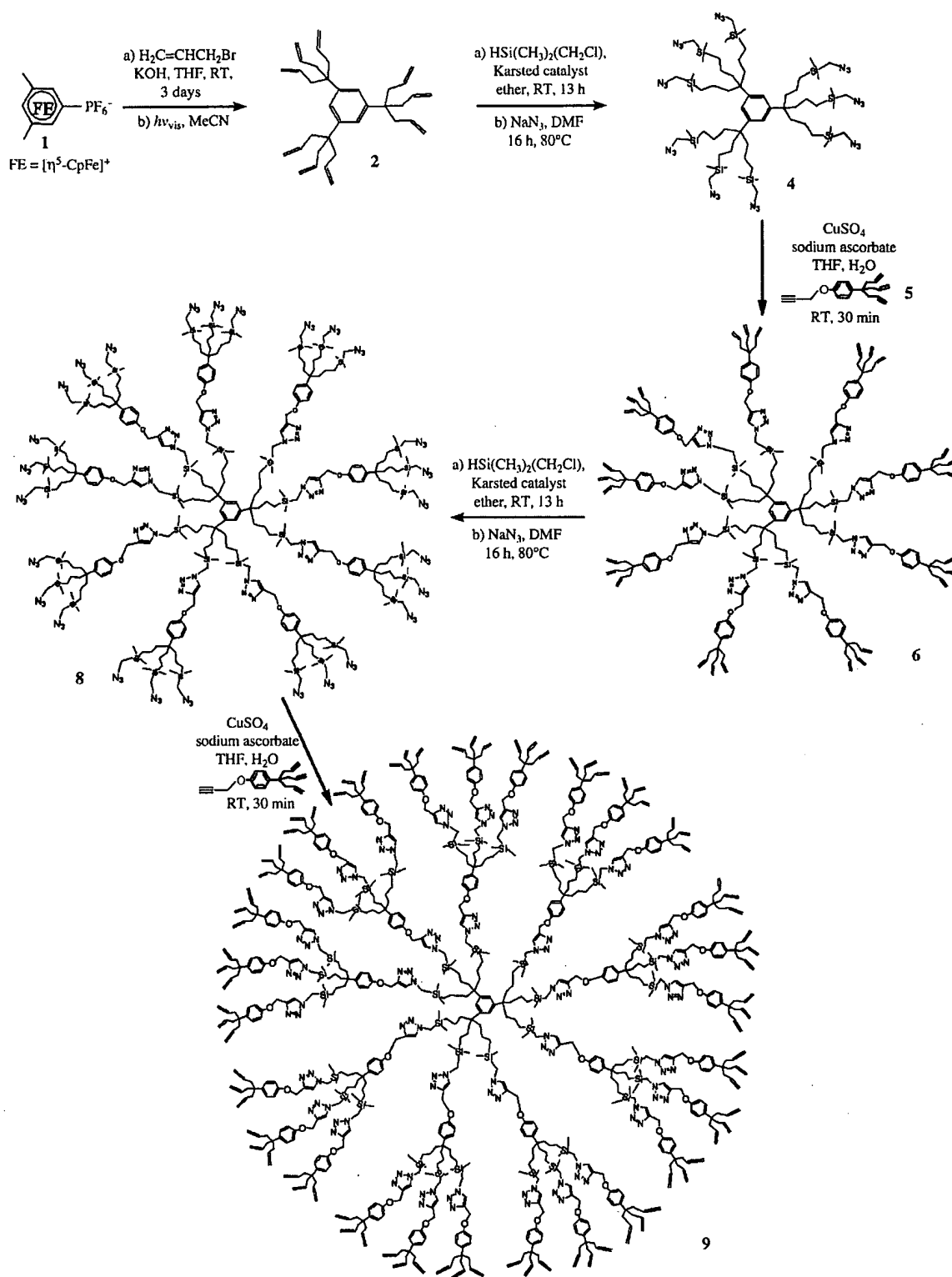
Scheme 1. Click synthesis of the 81-allyl dendrimer 9.

receptors[10] and dendritic *exo* receptors.[2b] Very recently, Beer et al. have developed systems able to recognize both cations and anions in ion pairs using this principle.[11] The group of van Koten has also reported the use of dendrimers for efficient sensing.[2a] The ferrocenyl dendrimers 12–14 recognize both oxo anions and transition-metal cations by means of a new CV wave which appears when an oxo anion ($H_2PO_4^-$ or ATP^{2-}, but not HSO_4^-) or a transition-metal cation (Cu^+, Cu^{2+}, Pd^{2+}, or Pt^{2+}) salt is added to an electrochemical cell containing a CH_2Cl_2 solution of the click dendrimer (Figure 1). This result is a sign of a relatively "strong redox recognition" according to the Echegoyen–Kaifer model; a modest recognition is indicated by only a shift of the initial CV wave.[12]

For oxo anions, the new wave appears at a less positive potential than the initial wave, thus indicating that the dendrimer–oxo anion assembly is easier to oxidize than the dendrimer alone, probably because the anion donates electron density to the redox center. On the other hand, for metal cations, the new wave appears at a more positive potential than the initial wave,[13] thus showing that the cation–dendrimer assembly is more difficult to oxidize than the dendrimer alone, probably because coordination of the triazole ligand to the metal cation after MeCN ligand substitution withdraws electron density from the redox center through the triazole bridge.

This recognition by the click dendrimers indicates a dramatic positive dendritic effect, as there is no new CV wave or shift of the CV wave upon addition of the oxo anion or metal cation when the non-dendritic monoferrocenyl click model 15 [Eq. (4)] is used instead of the click dendrimers, except for Pd^{2+}.

Figure 1. Redox sensing of both oxo anions (A⁻) and metal cations (M⁺) by poly-1,2,3-triazolylferrocenyl dendrimers: cyclic voltammograms of 12 ($c = 8.37 \times 10^{-5}$ M) a) without and b) in the presence of (nBu_4N)(H_2PO_4) (1 equiv per branch); c) cyclic voltammogram of 12 ($c = 1.26 \times 10^{-4}$ M) in the presence of [Pd(MeCN)$_4$](BF$_4$)$_2$ (0.25 equiv per branch). Fc*: [FeCp*$_2$], Cp* = C$_5$Me$_5$.

The recognition data are gathered in Table 1 (for the oxo anions), Table 2 (for Pd^{2+} and Pt^{2+}), and Table 3 (for Cu^+ and Cu^{2+}), which include the differences of potentials observed between the initial CV wave and the new CV wave ($\Delta E_{1/2}$), the potential difference between the new anodic and cathodic waves ($E_{pa} - E_{pc}$), and the ratios of apparent association constants ($K_{(+)}/K_{(0)}$).

For the oxo anions, the redox recognition with dendritic effect is also selective, that is, the appearance of a new wave with the click dendrimer 12 is the same with or without addition of the anions HSO_4^- or Cl^- at the same time as $H_2PO_4^-$ or ATP^{2-} (nBu_4N^+ salts). The stoichiometry of the titration corresponds to one equivalent of $H_2PO_4^-$ or half an equivalent of ATP^{2-} per ferrocenyl branch. Since *para-tert*-butylphenol (more acidic than $H_2PO_4^-$) is not

recognized by these dendrimers (no CV-wave shift), we conclude that H···N hydrogen bonding between such a

Table 1: Cyclic voltammetric data for compounds 12–15 before and after titration with (nBu_4N)$_2$(ATP) or (nBu_4N)(H_2PO_4) in CH_2Cl_2. All energy values are given in V.

$E_{1/2}$[a] ($E_{pa} - E_{pc}$)	i_{pc}/i_{pa}	n[b]	Recognition of ATP²⁻			Recognition of H₂PO₄⁻		
			$E_{1/2}$[a] ($E_{pa} - E_{pc}$)	$\Delta E_{1/2}$[c]	$K_{(+)}/K_{(0)}$[d]	$E_{1/2}$[a] ($E_{pa} - E_{pc}$)	$\Delta E_{1/2}$[c]	$K_{(+)}/K_{(0)}$[d]
15	0.570 (0.060)	1.0	1	0.560 (0.100)	0.010	1.5	0.560 (0.100)	0.010	1.5
12	0.555 (0.050)	1.2	9	0.400 (0.140)	0.155	470	0.355 (0.140)	0.200	2807
13	0.555 (0.040)	1.8	29	0.445 (0.130)	0.110	79	0.385 (0.120)	0.170	853
14	0.555 (0.050)	1.2	87	0.465 (0.120)	0.090	36	0.400 (0.115)	0.155	470

[a] $E_{1/2} = (E_{pa} + E_{pc})/2$ vs. [FeCp*$_2$]. The peak potentials might be perturbed by some adsorption,[9b] thus $K_{(+)}/K_{(0)}$ values must be considered with caution. Adsorption is weak, however, during the titration process and becomes important only at, and after, the equivalent point. The potential of the new wave does not vary significantly during the titration or at the equivalent point. Thus, errors in the $E_{1/2}$ values are limited. Electrolyte: 0.1 M (nBu_4N)(PF$_6$); working and counter electrodes: Pt; quasi-reference electrode: Ag; internal reference: [FeCp*$_2$]; scan rate: 0.200 Vs⁻¹; 20°C. [b] Number of electrons involved calculated from the Anson–Bard equation[9] using anodic intensities. [c] Difference between values of $E_{1/2}$ before and after titration. [d] Ratios of apparent association constants; error < 10%; $\Delta E_{1/2} = 0.058 \log(K_{(+)}/K_{(0)})$[12] at 20°C.

Table 2: Cyclic voltammetric data for compounds 12–15 before and after titration with $[Pd(MeCN)_4](BF_4)_2$ or $[Pt(MeCN)_4](BF_4)_2$ in CH_2Cl_2. All energy values are given in V.

$E_{1/2}$[a] ($E_{pa}-E_{pc}$)	Recognition of Pd^{2+}			Recognition of Pt^{2+}		
	$E_{1/2}$ ($E_{pa}-E_{pc}$)	$\Delta E_{1/2}$[b]	$K_{(0)}/K_{(+)}$[c]	$E_{1/2}$ ($E_{pa}-E_{pc}$)	$\Delta E_{1/2}$[b]	$K_{(0)}/K_{(+)}$[c]
15 0.570 (0.060)	0.715 (0.060)	−0.145	316	0.570 (0.060)	0	–
12 0.555 (0.050)	0.705 (0.050)	−0.150	386	0.665 (0.030)	−0.110	79
13 0.555 (0.040)	0.695 (0.040)	−0.140	259	0.655 (0.020)	−0.100	53
14 0.555 (0.050)	0.690 (0.035)	−0.135	213	0.650 (0.020)	−0.095	43

For [a], [b], and [c] see footnotes [a], [c], and [d] to Table 1, respectively.

Table 3: Cyclic voltammetric data for compounds 12–15 before and after titration with $[Cu(MeCN)_4](BF_4)$ or $[Cu(MeCN)_4](BF_4)_2$ in CH_2Cl_2. All energy values are given in V.

$E_{1/2}$[a] ($E_{pa}-E_{pc}$)	Recognition of Cu^{2+}			Recognition of Cu^+		
	$E_{1/2}$ ($E_{pa}-E_{pc}$)	$\Delta E_{1/2}$[b]	$K_{(0)}/K_{(+)}$[c]	$E_{1/2}$ ($E_{pa}-E_{pc}$)	$\Delta E_{1/2}$[b]	$K_{(0)}/K_{(+)}$[c]
15 0.570 (0.060)	0.570 (0.060)	0	–	0.570 (0.060)	0	–
12 0.555 (0.050)	0.645 (0.045)	−0.090	36	0.605 (0.030)	−0.050	7
13 0.555 (0.040)	0.635 (0.020)	−0.080	24	0.635 (0.020)	−0.080	24
14 0.555 (0.050)	0.625 (0.015)	−0.070	16	0.645 (0.015)	−0.090	36

For [a], [b], and [c] see footnotes [a], [c], and [d] to Table 1, respectively.

relatively acidic substrate and the triazole ring is insufficient to perturb the nearby ferrocenyl redox system. Thus, $H_2PO_4^-$ and ATP^{2-} ions are essentially recognized through the negatively charged oxygen atoms of the phosphato groups, which interact strongly with the Fe center when the latter is oxidized to Fe^{III} at the anode. Nevertheless, this $Fe^{\delta+}\cdots O^{\delta-}$ electrostatic perturbation of the redox center is presumably facilitated by chelation of the phosphato group through the above-mentioned N···H hydrogen bond (it is too weak in the neutral ferrocenyl form to provoke any signal shift in the ^1H NMR spectrum).

Contrary to all the previous redox recognition studies with both $H_2PO_4^-$ and ATP^{2-} ions and other metallodendrimers, we also observed a negative dendritic effect, that is, $\Delta E_{1/2}$ decreases slightly along the series $G_0 \to G_1 \to G_2$ for both oxo anions and metal cations, except for Cu^+ (see below). This negative dendritic effect could possibly be due to inhibiting steric effects.

The $E_{pa}-E_{pc}$ values illustrate the heterogeneous electron-transfer rates and adsorption events, and these values are completely different for oxo anions and metal cations. Whereas large $E_{pa}-E_{pc}$ values (140 mV for **12** and decreasing slightly with the dendrimer size) show the important reorganization of the dendrimer–guest assemblies upon slow heterogeneous electron transfer in the case of oxo anions,

small $E_{pa}-E_{pc}$ values (30–50 mV for **12** and decreasing with dendrimer size down to 15–35 mV), which are lower than the standard value of 58 mV at 25 °C for all the transition-metal cations, indicate some adsorption onto the electrode owing to the positive charges gained by the dendrimer–cation assemblies. The increased tendency of dendrimers to adsorb onto electrodes when their size increases can be exploited for the fabrication of dendrimer-derivatized electrodes (see Supporting Information, Figure S12) that are also useful for sensing.

There are marked differences in the redox recognition features of the selected transition-metal cations: Cu^+, Cu^{2+}, and Pt^{2+} are not recognized by the model complex **15**, and the redox recognition of Cu^+ ions shows a continuously positive dendritic effect as the dendrimer generation increases (Figure 2). On the other hand, the redox recognition for Pt^{2+} ions is optimal with G_0.

Compared to these marked dendritic effects, there is no significant dendritic effect for Pd^{2+} ions, which is recognized by the model complex **15** as well as by the dendrimers with approximately the same $\Delta E_{1/2}$ values. This result shows that, of all the metal ions studied, Pd^{II} clearly has the strongest affinity with the 1,2,3-triazole ligand.[13] The titrations reveal a stoichiometry of two 1,2,3-triazole ligands per Pd^{II} (model
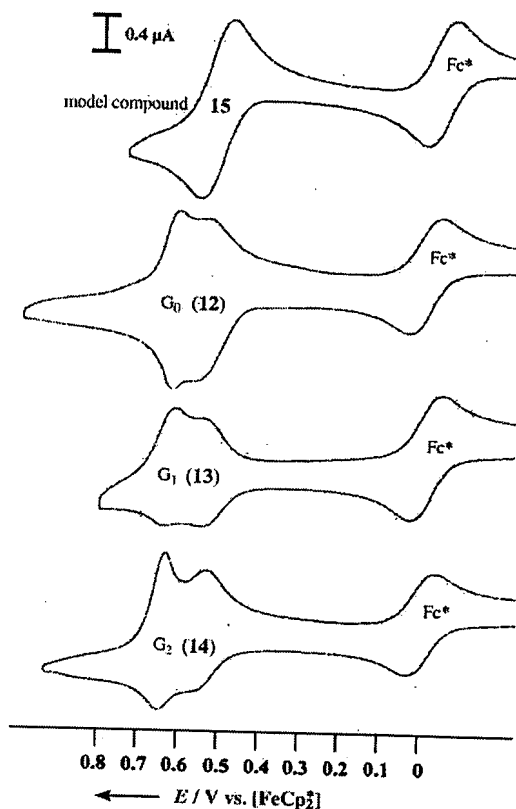
Figure 2. Positive dendritic effect in the titration of Cu^+ ions with dendrimers 12–14 compared to the model compound 15. Cyclovoltammograms of 15 (1.33×10^{-3} M; 1 equiv Cu^+ per branch), 12 (1.26×10^{-4} M; 0.11 equiv Cu^+ per branch), 13 (2.70×10^{-5} M; 0.11 equiv Cu^+ per branch), and 14 (9.0×10^{-6} M; 0.11 equiv Cu^+ per branch). (See Supporting Information for experimental conditions.)

15), although this increases for higher dendrimer generations. This trend also depends on the nature of the metal. For instance, with [Pd(MeCN)₄](BF₄)₂ in CH₂Cl₂ the stoichiometry reaches four for **12**, whereas with [Cu(MeCN)₄](BF₄) it is only two for **12**. It is likely that the synergy between the steric constraints and increased number of potential ligands as the dendrimer generation increases disfavors chelation by the 1,2,3-triazole ring and favors the binding of four triazole ligands to each metal center in the large dendrimers. This trend is clearly more drastically marked with the planar geometry of Pd^{II} complexes than with the tetrahedral geometry of Cu^{I} complexes, which can more easily accommodate the three-dimensionality of the intradendritic confinement.

In conclusion, we have synthesized the first click metallodendrimers and shown their use as redox sensors that allow the selective recognition of both oxo anions (H₂PO₄$^-$ and ATP^{2-}) and transition-metal cations with a variety of dramatic dendritic effects.[14] Finally, we show that these easily synthesized composite metallodendrimers with multiple internal heterocyclic ligands clearly offer attractive possibilities for encapsulation, transport, and catalysis.

Experimental Section

General procedure for the click reactions: The azido dendrimer (1 equiv) and the alkyne (1.5 equiv per branch) were dissolved in THF and water was added (1:1, THF/water). At 20°C, CuSO₄ (1M aqueous solution, 1 equiv per branch) was added then a freshly prepared solution of sodium ascorbate (1M aqueous solution, 2 equiv per branch) was added dropwise. The solution was then stirred for 30 min at room temperature. After removing THF under vacuum, dichloromethane and an aqueous solution of ammonia were added. The mixture was stirred for 10 min to remove all the Cu^{I} trapped inside the dendrimer as [Cu(NH₃)₆]$^+$ ions. The organic phase was washed twice with water, filtered through celite, and the solvent was removed under vacuum. The product was washed with pentane to remove the excess of alkyne and precipitated by addition of dichloromethane/pentane. The organic dendrimers were obtained as colorless, waxy products and the ferrocenyl dendrimers were obtained as orange, waxy products, usually in high yields in both series. For details of all experimental procedures, characterization data of all products, mass spectra, ¹H NMR spectra, cyclic voltammetry conditions, and voltammograms monitoring the titrations, see the Supporting Information

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'Click' Bioconjugation of a Well-Defined Synthetic Polymer and a Protein Transduction Domain

Jean-François Lutz,[A,C] *Hans G. Börner,*[B] *and Katja Weichenhan*[A]

[A] Research Group Nanotechnology for Life Science, Fraunhofer Institute for Applied Polymer Research, Geiselbergstrasse 69, Potsdam-Golm 14476, Germany.

[B] Max Planck Institute of Colloids and Interfaces, Colloid Department, 14424 Potsdam-Golm, Germany.

[C] Corresponding author. Email: lutz@iap.fhg.de

The copper-catalyzed 1,3-dipolar 'click' cycloaddition of azides and alkynes was studied to link a model synthetic polymer to a sequence-defined protein transduction domain (PTD). The bromine chain-ends of a well-defined polystyrene (PS) sample synthesized by atom transfer radical polymerization (M_n 2200 g mol^{-1}, M_w/M_n 1.21) were first transformed into azide functions by substitution with sodium azide, and subsequently reacted with an alkyne-functionalized PTD (i.e., the oligopeptide sequence GGYGRKKRRQRRRG, also known as the TAT peptide). The click bioconjugation proceeded successfully at room temperature, thus affording the targeted PS-*b*-GGYGRKKRRQRRRG bioconjugate in high yields. However, a slight molar excess of polystyrene was required for optimal coupling.

Within the last two years, 'click' chemistry has become a very popular tool in synthetic polymer science.[1,2] The term 'click' was first introduced by K. Barry Sharpless in early 2001 and refers to almost perfect organic reactions, which are very efficient, highly selective, and require only mild experimental conditions.[3] Although several organic transformations may be ranked as click reactions, the copper-catalyzed 1,3-dipolar cycloaddition of terminal alkynes and azides is generally regarded as the archetype of click chemistry.[4,5] This particular reaction has been recently demonstrated to be an efficient and versatile tool for preparing or modifying tailor-made macromolecules such as conjugated polymers, block copolymers, rotaxanes, miktoarm stars, graft copolymers, dendrimers, or networks.[1,2,6–12] Moreover, copper-catalyzed azide–alkyne cycloadditions (CuAAC) possess the important advantage of proceeding at room temperature, in relatively benign solvents (including water), and in the presence of many other functional groups. Hence, these reactions are also particularly suitable for modifying complex materials such as fragile supramolecular assemblies or multifunctional biological structures.[1] This latter aspect is particularly topical since bio-hybrid structures made of synthetic and biological moieties have lately gained interest in many applied areas of materials science.[13] Thus, CuAAC have been recently investigated for linking synthetic polymers, dendrimers, or inorganic nanoparticles to biological structures such as carbohydrates, nucleic acids, proteins, enzymes, viruses, or cells.[14–20]

In this context, we[21] and others[17,22,23] have reported the click ligation of synthetic macromolecules with sequence-defined oligopeptides. Polymer–peptide bioconjugates constitute a promising new class of macromolecules, which combine the advantages of standard synthetic polymers with advanced biological functions (e.g., molecular recognition, programmed self-organization, biological targeting).[13] For example, in our previous studies, a well-defined biocompatible poly(oligoethylene glycol acrylate) segment[24] has been successfully coupled to the RGD peptide (i.e., a cell adhesion motif recognized by integrin receptors) using CuAAC.[21] The aim of the present communication is to further demonstrate the efficacy of CuAAc for coupling synthetic polymers and sequence-defined oligopeptides.

Protein transduction domains (PTDs), also known as cell penetrating peptides (CPPs), are short oligopeptides (i.e., sequences typically shorter than 30 amino acids) with a high content of cationic amino acids (e.g., arginine and lysine) and, therefore, have a strong ability to cross cellular membranes.[25] The most studied PTD is undoubtedly the short arginine-rich sequence GRKKRRQRRR, which corresponds to the amino acid residues 48–57 of the TAT protein. This particular sequence has been frequently used for enhancing the intracellular delivery of either low-molecular-weight drugs, proteins, oligonucleotides, or DNA plasmids.[26] Thus, the controlled bioconjugation of TAT to synthetic materials is an important issue for designing efficient intracellular nanocarriers. However, so far only a few research groups have reported detailed pathways for preparing TAT–polymer bioconjugates.[27,28] For instance, Kissel and co-workers studied a vinyl sulfone/thiol coupling approach to link poly(ethyleneimine)-*block*-poly(ethylene glycol) copolymers and TAT.[28] Alternatively, Wooley and co-workers used an alkoxyamine-functionalized TAT as a macroinitiator for nitroxide-mediated polymerization (NMP).[27] Their synthetic strategy relied on the solid-phase supported synthesis of the sequence-defined oligopeptide followed by two modification steps to introduce the alkoxyamine moiety. Subsequently, the
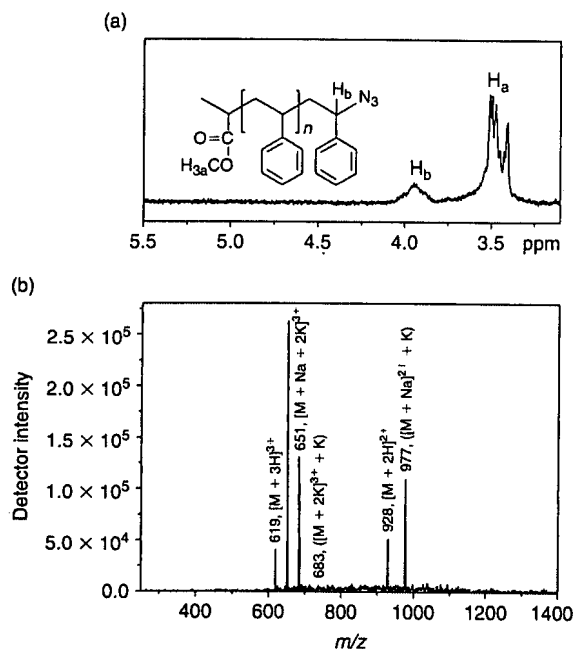
Fig. 1. (a) ^{1}H NMR spectrum (zoom of the region 5.5–3 ppm) recorded in CDCl$_3$ for the purified ω-azido PS. (b) Mass spectrum (ESI) of the fully deprotected alkyne-functionalized TAT oligopeptide.

oligopeptide macroinitiator was used directly to initiate solid-phase supported NMP of alkyl acrylates.

The click ligation of an alkyne-functionalized TAT sequence and an azido-functionalized polymer was studied. Polystyrene (PS) was selected as a model polymer because of its ease of handling. A well defined polystyrene sample was prepared by atom transfer radical polymerization (ATRP) using methyl 2-bromopropionate as an initiator and a combination of copper(I) bromide and N,N,N',N',N'-pentamethyldiethylenetriamine as catalyst.[7,29] After reaction and purification, the polymer was characterized by size exclusion chromatography (SEC, number-average molecular weight M_n 2200 g mol^{-1}, molecular weight distribution M_w/M_n 1.21) and ^{1}H NMR spectrosccopy. The latter technique indicated that the polymer possessed $\sim$85% of bromine ω-end-groups, which is in good agreement with previously reported values.[7,30,31] It is important to remember that this number cannot be 100% since ATRPs are, by essence, subject to termination reactions.[30,31] The bromo-functional PS was subsequently transformed into an azide ω-functionalized polymer by nucleophilic substitution in the presence of sodium azide. As previously reported, this reaction was found to be quantitative.[7] The final PS contained $\sim$85% of ω-azido end-groups, as confirmed by ^{1}H NMR spectroscopy (Fig. 1a).

The TAT oligopeptide GGYGRKKRRQRRRG was prepared by solid-phase supported synthesis using standard Fmoc protection chemistry. While still on the solid support, the N-terminus of the oligopeptide was transformed into an alkyne moiety by a coupling reaction with 4-pentynoic acid facilitated by N,N'-dicyclohexylcarbodiimide. Subsequently, the peptide was cleaved from the solid support and purified. The molecular structure of the alkyne-functionalized TAT peptide was confirmed by mass spectrometry as shown on Fig. 1b. This particular measurement was performed on a fully deprotected sample (M 1853 g mol^{-1}) since protecting side-groups prevent effective

desorption and stabilization of ions in the electrospray ionization (ESI) process. However, for the CuAAC with PS, the protecting groups of tyrosine, arginine, lysine, and glutamine residues (i.e., t-butyl, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl, t-butyloxycarbonyl, and trityl, respectively) were retained in the molecular structure (Scheme 1, M 3864 g mol^{-1}). Such a precaution is theoretically not necessary since, as aforementioned, CuAAC is a highly chemoselective reaction. Several studies have demonstrated that unprotected peptides can be directly used in click reactions.[17,22,23] However, the main target of the present model study is to investigate the efficiency of CuAAC for macromolecular coupling, rather than to explore the properties of the formed bio-hybrid structure. Thus, the protecting side-groups were preserved to allow sufficient solubility of the peptide segment in organic solvents and, therefore, a reliable SEC analysis of the polymer bioconjugate. It has been previously demonstrated that oligopeptides may be deprotected after performing a CuAAC and that triazole rings are generally not damaged in concentrated trifluoroacetic acid (TFA).[32,33]

The click reaction was investigated in solution in N-methyl pyrrolidone in the presence of Cu^{I}Br and 2,2'-bipyridyl. A stoichiometric ratio of azide to alkyne was first investigated (i.e., 1 equiv. of the peptide with 100% alkyne functionality and 1.17 equiv. of the PS with 85% of azide functionality). After 24 h of reaction, the targeted diblock copolymer PS-b-TAT ($M_{n\,th}$ $\sim$6000 g mol^{-1}) could be observed by SEC. However, the coupling was not quantitative since a trimodal chromatogram was measured (data not shown). In addition to the expected excess of PS (i.e., 0.17 equiv.) and the diblock, a peak of unreacted TAT was also observed, which indicated a yield of coupling not higher than 70–80%.

In order to achieve higher yields of coupling, a slightly larger excess of PS (i.e., 1.5 equiv.) was used in the click reaction. Fig. 2 shows the SEC chromatogram measured for this reaction after one day at room temperature. In that case, only two peaks, which correspond to the excess PS (i.e., 0.5 equiv.) and the formed diblock, were measured. The TAT oligopeptide could not be detected by SEC, which indicates a high coupling yield. Yet, such a coupling strategy that uses excess quantities of one reactant requires further purification (e.g., preparative SEC or membrane filtration) if a well-defined diblock copolymer is wanted. However, this method can also be applied in a straightforward manner to modify the surfaces of preformed nanomaterials (e.g., for functionalizing ATRP polymer brushes grown on flat or colloidal surfaces).

In conclusion, click chemistry has been investigated as a direct ligation tool for the bioconjugation of a model synthetic polymer with a sequence-defined oligopeptide. Although a quantitative coupling could not be obtained with stoichiometric amounts of reactants, high yields were reached when a slight excess of PS was used. These new data further confirm that CuAAc is a facile and versatile reaction for polymer bioconjugation.

Experimental

Synthesis of the Alkyne-Functionalized Oligopeptide GGYGRKKRRQRRRG (N-(4-Pentynamide)-TAT Peptide)

The solid-phase supported peptide synthesis of the TAT peptide was performed on an ABI 433a peptide synthesizer (Applied Biosystems, Germany) using N-methyl-2-pyrrolidone (NMP) as solvent. The sequential coupling of the Fmoc-protected amino acid derivatives (IRIS Biotech GmbH,
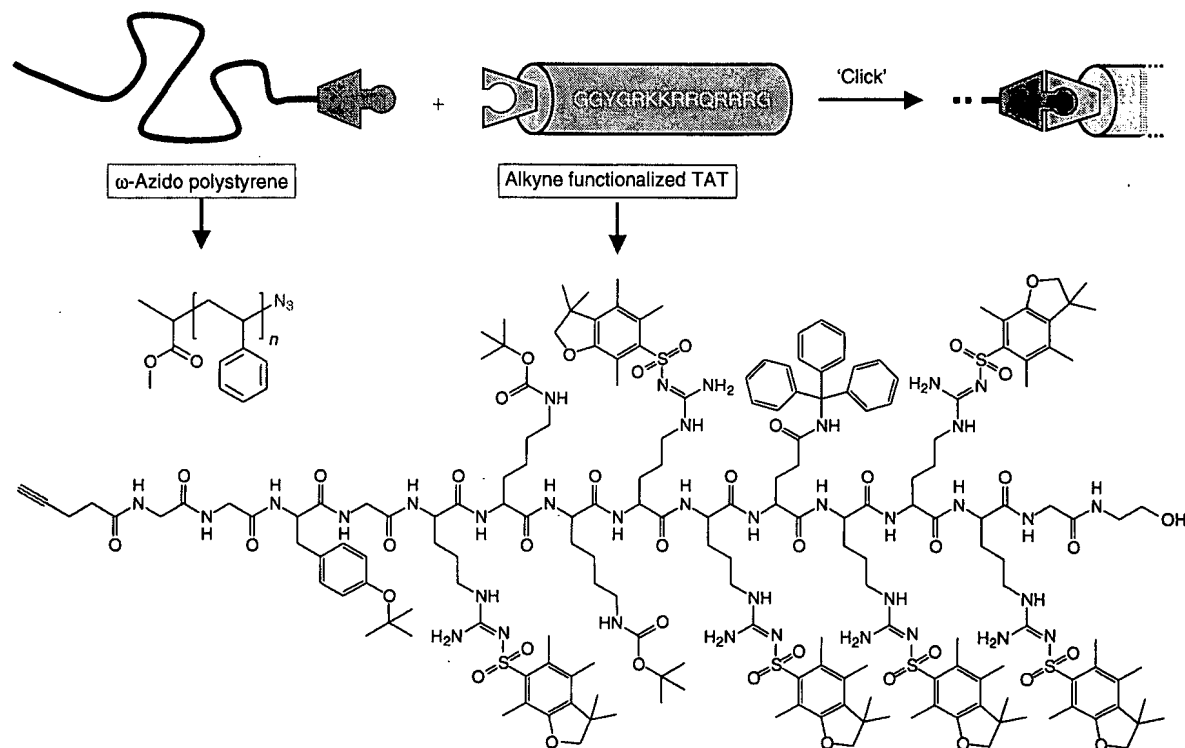
Scheme 1. 'Click' synthetic strategy studied herein for coupling an alkyne-functionalized TAT polypeptide and well-defined PS having an ω-azido end-group.
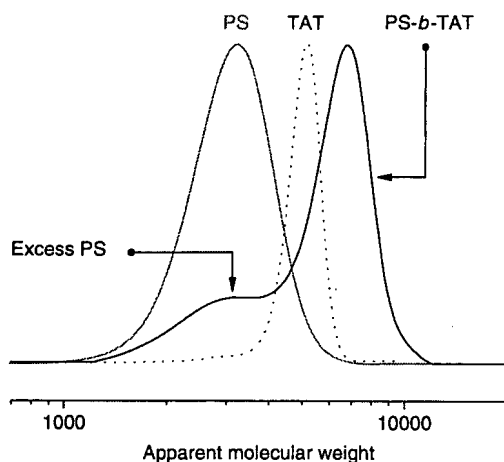
Fig. 2. SEC chromatograms measured in NMP for the ω-azido PS (full red line), the protected alkyne-functionalized TAT oligopeptide (blue dotted line), and the resulting bioconjugate PS-b-TAT (full black line).

Germany) was facilitated by HBTU (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)/DIPEA (N,N'-diisopropylethylamine), and standard FastMoc protocols (Applied Biosystems, Germany) were followed as described previously.[34,35] A PS-(2-chlorotrityl chloride) resin was preloaded with 9H-fluoren-9-ylmethyl N-(2-hydroxyethyl) carbamate (Fmoc-N-(2-aminoethanol)) using standard procedures and subsequently used to attach the Fmoc-protected amino acid derivatives to yield the resin-bound peptide: Fmoc-GGYGRKKRRQRRRG–NHCH₂CH₂O-resin. After removal of

the Fmoc protecting group, the N-terminal amine was amidated with 20 equiv. excess of 4-pentynoic acid using standard DCC (dicyclohexylcarbodiimide) coupling protocols.[34] The alkyne-functionalized TAT sequence was subsequently liberated from the support by treating twice with a cleavage mixture (2 vol.% of TFA in dichloromethane) for 2 min, followed by three washing cycles. After addition of 50 mL of dioxan the cleavage solution was concentrated under vacuum, followed by diethyl ether precipitation, centrifugation, and lyophilization from dioxan. The alkyne-functionalized TAT sequence (with protected tyrosine, arginine, lysine, and glutamine units, Scheme 1) was isolated in ~67% yield without additional purification by HPLC. ESI (of the fully deprotected peptide, deprotection conditions: 94:5:1 vol.% of TFA:H₂O:triisopropylsilane for 2 h): m/z 977 ([M + Na]²⁺ + K), 928 ([M + 2H]²⁺), 683 ([M + 2K]³⁺ + K), 651 ([M + Na⁺ + 2K]³⁺), 619 ([M + 3H]³⁺). $C_{77}H_{136}N_{36}O_{18}$, M_{th} 1853.08 Da.

Synthesis of the ω-Azido PS

The full procedure for the synthesis of well-defined PS with a terminal azide functionality was described in detail in a previous report.[7] Typically, a bromine end-functionalized PS (prepared using ATRP, M_n 2200 g mol⁻¹, 4.5 g, 2 mmol), sodium azide (146 mg, 2.2 mmol), and N,N-dimethylformamide (9 mL) were added in a flask. The clear homogeneous solution was stirred at room temperature for 3 h. After reaction, the PS was precipitated into methanol, filtered, and dried under vacuum. ¹H NMR analysis of the purified polymer indicated a quantitative transformation of the bromine end-group into an azide function.

Example of Click Ligation of Azide-Functionalized PS and Alkyne-Functionalized TAT Peptide

PS (85 mg, 0.038 mmol), TAT peptide (100 mg, 0.025 mmol), and copper bromide (37 mg, 0.25 mmol) were added to a dry flask. The glassware was capped with a septum and purged with dry argon for several minutes. 2,2′-Bipyridyl (82 mg, 0.5 mmol) dissolved in 2.5 mL of degassed NMP was then added by a degassed syringe. The mixture turned dark brown, which indicated complexation of Cu^IBr and 2,2′-bipyridyl. The reaction mixture was stirred for 24 h at room temperature. After the reaction, the copper catalyst was removed by filtration on a silica column and the resulting block copolymer solution in NMP was directly studied by SEC.

Measurements and Analysis

Size Exclusion Chromatography

Molecular weights and molecular weight distributions were determined by SEC performed at 70°C in NMP (flow rate: 0.8 mL min^{-1}), using two PSS-GRAM columns (Polymer Standards Service GmbH) with a particle size of 7 μm and a porosity of 100 and 1000 Å (the porosity values do not correspond to real pore diameters but to manufacturer standards). The detection was performed with an RI- (Shodex RI-71) and a UV-detector (TSP UV 1000; 260 nm). For calibration, linear PS standards (Polymer Standards Service GmbH) were used.

Mass Spectrometry

Mass spectra were recorded on a Shimadzu QP8000 electrospray mass spectrometer (Shimadzu, Germany) with N_2 as a carrier gas (4.5 L min^{-1}), an injector temperature of 150°C, and an acceleration voltage of 4.5 kV. The 0.1 mg mL^{-1} samples in methanol with 0.1% formic acid were injected and the detection proceeded in the positive mode with a detector voltage of 1.6 kV.

^{1}H NMR Spectroscopy

^{1}H NMR spectra were recorded in CDCl$_3$ on a Bruker DPX-400 operating at 400.1 MHz.

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'Click' Chemistry in Polymer and Materials Science

Wolfgang H. Binder,* Robert Sachsenhofer

The modification of polymers after the successful achievement of a polymerization process represents an important task in macromolecular science. Cycloaddition reactions, among them the metal catalyzed azide/alkyne 'click' reaction (a variation of the Huisgen 1,3-dipolar cycloaddition reaction between terminal acetylenes and azides) represents an important contribution towards this endeavor. They combine high efficiency (usually above 95%) with a high tolerance of functional groups and solvents, as well as moderate reaction temperatures (25–70 °C). The present review assembles recent literature for applications of this reaction in the field of polymer science (linear polymers, dendrimers, gels) as well as the use of this and related reactions for surface modification on carbon nanotubes, fullerenes, and on solid substrates, and includes the authors own publications in this field. A number of references (>100) are included.
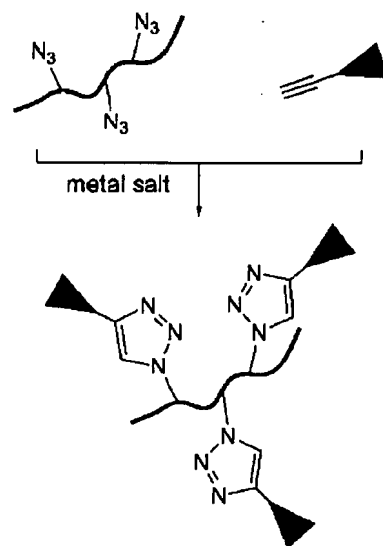
Introduction

The azide/alkyne 'click' reaction[1] (also termed the Sharpless 'click' reaction) is a recent re-discovery of a reaction fulfilling many requirements for the affixation of ligands onto polymers by post-modification processes, which include a) often quantitative yields, b) a high tolerance of functional groups, c) an insensitivity of the reaction to solvents, irrespective of their protic/aprotic or polar/non-polar character, and d) reactions at various types of interfaces, such as solid/liquid, liquid/liquid, or even solid/solid interfaces. The present review focuses on issues related to the reaction itself as well as on the wide applications in polymer science, material, and surface science.

The basic reaction, which is nowadays summed up under the name 'Sharpless-type click reaction', is a variant of the Huisgen 1,3-dipolar cycloaddition reaction[2a,2b] between C—C triple, C—N triple bonds, and alkyl-/aryl-/sulfonyl azides (see Scheme 1).

The relevant outcomes of this reaction are tetrazoles, 1,2,3-triazoles, or 1,2-oxazoles (Scheme 1a–c, respectively). Besides the 1,3-dipolar cycloaddition reaction, classical Diels-Alder-type reactions (Scheme 1d) have been used extensively for the functionalization of polymeric materials and surfaces.[3] These reactions are located within a series of reactions named click reactions, which is defined by a gain of thermodynamic enthalpy of at least 20 kcal·mol⁻¹,[1] thus leading to reactions characterized by high yields, simple reaction conditions, fast reaction times, and high selectivity. Among many reactions tested, the 1,3-dipolar cycloaddition process has emerged as the method of choice to effect the requirements of ligating two

W. H. Binder, R. Sachsenhofer
Vienna University of Technology, Institute of Applied Synthetic Chemistry/Division of Macromolecular Chemistry, Getreidemarkt 9/163/MC, A-1060 Vienna, Austria
E-mail: wbinder@mail.zserv.tuwien.ac.at

Wolfgang H. Binder is currently associate professor of chemistry at the ViennaUniversity of Technology. After finishing his studies with a PhD in organic carbohydrate chemistry from the University of Vienna (Austria) with Prof. W. Schmid in 1995 he spent a PostDoc with Prof. F. M. Menger on combinatorial polymer chemistry and subsequently spent another PostDoc with Prof. J. Mulzer (University of Vienna) in total organic synthesis. Since 2004 he is associate professor at the University of Technology leading a group in polymer chemistry. His current research interests are centered on macromolecular synthesis, nanotechnology and nanoparticles, with a focus on block copolymers, self assembled monolayers, supramolecular chemistry and membranes.

Dipl.Ing. Robert Sachsenhofer is currently a Ph.D.-student at the Vienna University of Technology, Austria. After studying chemistry in Vienna, he joined the group of Prof. Binder for a diploma-thesis in the field of luminescent nanoparticles and their surface modifications via "click"-type reactions. After finishing his Diploma in chemistry in 2005, he joined the group of Prof. Binder for a PhD-thesis in the field of self assembly and "click"-reactions on lyotropic phases.

molecules in a general, fast, and efficient process. Critical for the broad application of the reaction as a click-type reaction is the discovery that the central, purely thermal Huisgen process can be extremely accelerated by the

addition of various metal species (Ru, Ni, Pt, Pd), but mostly by Cu^I species, within the reaction system (see Scheme 2).

Whereas the purely thermal 1,3-dipolar cycloaddition reaction between aryl/alkyl azides and strongly activated alkynes (i.e., acyl- and sulfonyl cyanides as well as acyl-alkynes) was proposed (and investigated) by Sharpless and co-workers in 2001[1,4] as a click-type reaction, Meldal and co-workers published a paper in 2002[5] that describes the acceleration of this process by Cu^I salts, which leads to a reaction at 25 °C in quantitative yields, first mentioning the higher regioselectivity (1,4-triazole formation versus 1,5-triazole) with respect to the purely thermal process. Later, Sharpless and co-workers published a paper in 2002[6a] where the formation of 1,2,3-triazoles by the Cu^I-catalyzed Huisgen reaction between non-activated alkynes and alkyl/aryl azides was described. A catalytic cycle based on a concerted mechanism via a Cu acetylide intermediate was proposed, which has been recently revised to include a binuclear reaction mechanism on the basis of several observations.[6b] The critical 'invention' of this process is the transformation of a purely thermal 1,3-dipolar cycloaddition process to a 1,3- dipolar cycloaddition process catalyzed by metal salts (mostly Cu^I salts, but recently also Ru, Ni, Pd, and Pt salts) which runs at ambient temperature, is nearly solvent insensitive, and with an extremely high tolerance of functional groups. In the following chapter, a short mechanistic review will precede the recent applications of this reaction in various fields of science, most of all materials science, polymer chemistry, and biological applications. A survey of mostly recent literature related to the polymer science and
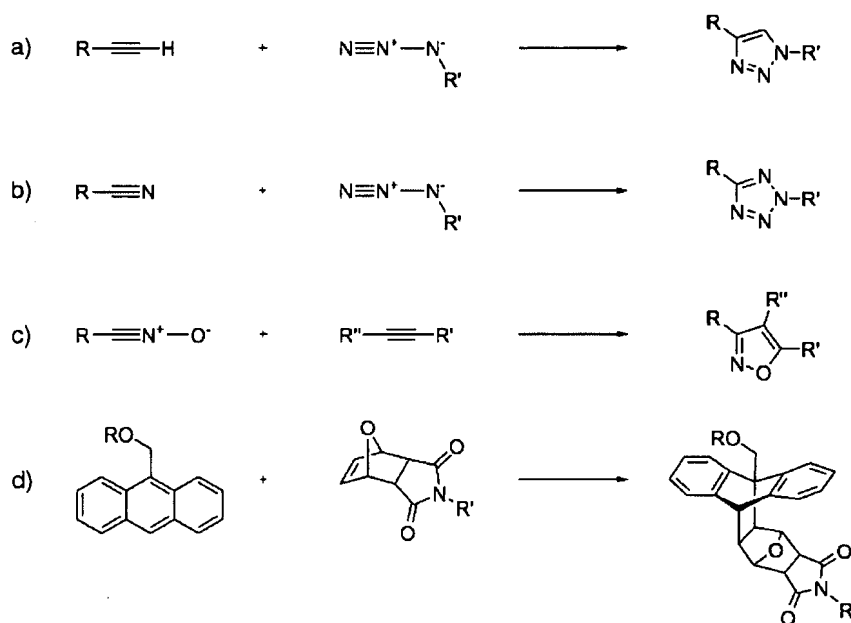
Scheme 1.
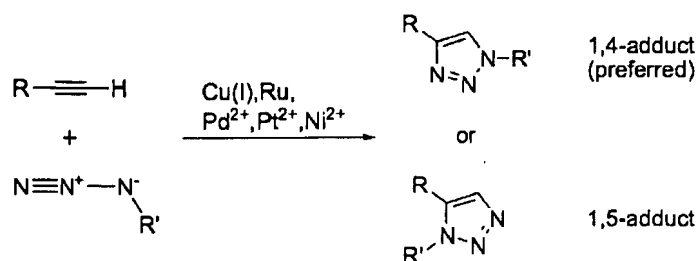
■ *Scheme 2.*

materials fields will draw a line to other previous reviews[1,7–9] describing the azide/alkyne click reaction,[1] in general, for application in organic chemistry,[8] as well as for drug discovery and biolabeling.[9]

Mechanistic Details/Catalysts

The basic process of the Huisgen 1,3-dipolar cycloaddition[2,10,11] is depicted in Scheme 2, generating 1,4- and 1,5-triazoles, respectively. Nearly all functional groups are compatible with this process, except those that are a) either self reactive, or b) able to yield stable complexes with the Cu^I metal under catalyst deactivation. Thus the functional groups depicted in Scheme 3a are not compatible with the azide/alkyne-type click reaction, placing the thermal Huisgen 1,3-dipolar cycloaddition process as the most important side reaction. The main interfering functional groups are strongly activated azides (i.e., acyl- and sulfonyl azides) as well as cyanides, which are able to compete in purely thermal cycloaddition processes (Scheme 3a).

Double bonds are tolerated to a certain extent, given that they are neither electronically activated (i.e., by electron withdrawing substituents) nor embedded into substrates of appropriate ring strain.[12] Thus the reactivity of phenylacetylene with phenylazide (reaction rate $k = 0.29 \times 10^7 \, dm^3 \cdot M^{-1} \cdot s^{-1}$) is comparable to that of hept-1-ene, styrene, and isoprene. Other alkenes and cycloalkenes display faster reactions with phenylazide (e.g.,

norbornene $k = 188 \times 10^7 \, dm^3 \cdot M^{-1} \cdot s^{-1}$, N-phenyl maleimide: $k = 27.60 \times 10^7 \, dm^3 \cdot M^{-1} \cdot s^{-1}$, and acetylene carbonic acid dimethyl ester : $k = 25.40 \times 10^7 \, dm^3 \cdot M^{-1} \cdot s^{-1}$). Therefore, the reaction with strained and electronically activated alkenes represents an important competitive reaction partner in the thermal process. Similar reasoning is also applicable for the rate-accelerating effect of electron-withdrawing substituents on the azido molecule as determined by Hammett correlations.[13]

A variety of catalytic systems have been used to effect the 1,3-dipolar cycloaddition process. In case of the azide/acetylene process, mostly Cu^I catalysts have been used (see Scheme 3b) and require about 0.25–2 mol-% of the catalysts. Most methods use Cu^I salts directly, other methods generate the copper(I) species by reduction of Cu^{II} salts using sodium ascorbate or metallic copper. Recently, the use of copper clusters of Cu/Cu oxide nanoparticles, sized 7–10 nm,[14] as well as copper clusters of diameter ≈ 2 nm, with a specific surface area of $168 \, m^2 \cdot g^{-1}$[15] have been described, although with contradicting explanations for their catalytic activity. Whereas in the former case the presence of Cu^I/Cu^{II} species in a ratio of 1:3 was made responsible for the strong catalytic activity, the latter publication claims the catalytic activity of the Cu^0 species. Both publications claim a positive influence of the alkylamine ligand present on the nanoparticle surface as an additional factor for activity enhancement. Besides the copper catalyst, 1–5 equivalents of base are added, mechanistically for promoting the formation of the copper(I)-acetylide. Solvents and bases are listed in Scheme 3b/3c, which features most of the known solvents as applicable for this reaction. In addition, biphasic reaction systems (water/alcohol or water/toluene) can be applied with excellent results. The bases used (Scheme 4) are mostly triethylamine, 2,6-lutidine, and N,N-diisopropylethylamine (DIPEA), as well as N,N,N', N',N''-pentamethylethylenetetramine (PMDETA) or 2,2'-bipyridine. Besides the copper(I) catalyst

a)
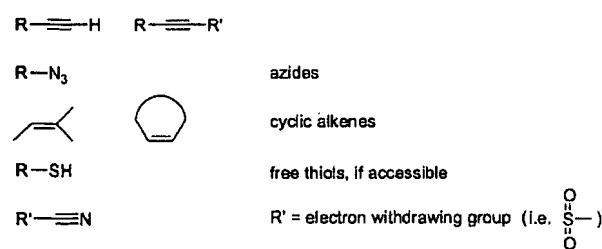
b)

catalysts

- $Cu(II)SO_4.5H_2O$ / sodium ascorbate
- $Cu(II)SO_4.5H_2O$ / copper metal
- $Cu(OAc)_2$ /sodium ascorbate
- $Cu(I)(MeCN)_4PF_6$
- $Cu(I)X$ X = Br, I
- $Cu(I)OTf.C_6H_6$
- $Cu(I)(PPh_3)_3Br$
- copper metal / NEt_3
- copper metal / $Cu(II)SO_4$ / microwave
- copper cluster
- Ru-cp* complexes
- Ni^{2+}
- Pd^{2+}
- Pt^{2+}

c)

solvents

- hexane, toluene, toluene/water
- butanol/water, alcoholic solvents
- halogenated solvents (CH_2Cl_2, $CHCl_3$)
- DMF, DMSO
- THF, diethylether

■ *Scheme 3.*
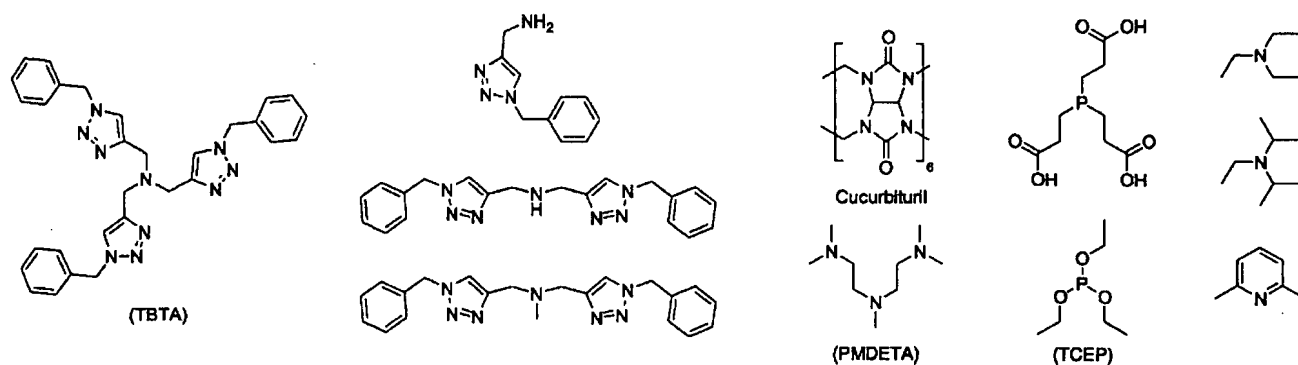
■ *Scheme 4.*

and the base, triazoles have been shown to accelerate the reaction.[16a] Some of the ligands are indicative of a complexation of the copper(I), which leads to a stabilization of the copper(I) oxidation state, thus prevents coupling reactions such as the Ullman,[17] and Cadiot-Chodkiewizc couplings.[18a] Moreover, tris-triazolyl ligands inhibit the Cu[II]-catalyzed oxidative coupling reactions of terminal alkynes to diynes under otherwise standard conditions.[18b] Other systems use tris(carboxyethyl)phosphine (TCPE) as a ligand. Recently, the systematic investigation of catalytic systems for the Sharpless click reaction has been achieved by parallel methods by a fluorescence-quenching assay.[16b] It should be mentioned that besides Cu[I] salts, other metals that promote the dipolar cycloaddition reaction of terminal acetylenes and azides have been reported recently. Thus Sharpless and co-workers found that a variety of Ru complexes (CpRuCl(PPh₃), [Cp*RuCl₂]₂, Cp*RuCl(NBD), and Cp*RuCl(COD)) promote the azide/alkyne click reaction.[19a] Interestingly, not only the 1,4-aduct is favored by some catalysts (i.e., Ru(OAc)₂(PPh₃)₂), but also the 1,5-adducts by other Ru catalysts. A catalytic cycle that relies on a pathway similar to the cyclotrimerization reaction of alkynes via a six-membered ruthenacycle has been proposed. Matyjaszewski and co-workers have recently published the use of Ni, Pd, and Pt salts to catalyze this reaction, although a mechanistic description is not provided.[19b]

Strong effects of alternative synthetic methodologies have been observed within the click reaction. Thus Fokin and co-workers [20] and others[21] have investigated the reaction under action of microwave irradiation. They used a combination of nucleophilic displacements starting from benzylic halides and sodium azide directly linking the present phenylacetylene in a single step. The reaction was strongly enhanced by the action of microwave irradiation, and furnished the corresponding triazoles in yields between 86–93%. Similar observations have been described with click reactions on polymers and dendrimers. Another approach[22] starts from the initial observation

that ammonium salts can act as co-catalysts for the 1,3-dipolar cycloaddition process when using solely metallic copper as the catalyst. Thus ammonium salts directly promote the formation of Cu[I] species, which enables an efficient reaction to yield the cycloaddition products in >95% yields.

A mechanistic picture of the copper catalyzed reaction was first proposed by Meldal and co-workers [5] and Sharpless and co-workers,[6] and has later been verified by computational methods.[23,24] However, the proposed catalytic mechanism (calculated[23] by density functional theory (DFT) calculations) that relied on the initial formation of a Cu acetylide between the Cu[I] species and the terminal alkyne which subsequently proceeded by an initial π-complex formation between the Cu[I] and the
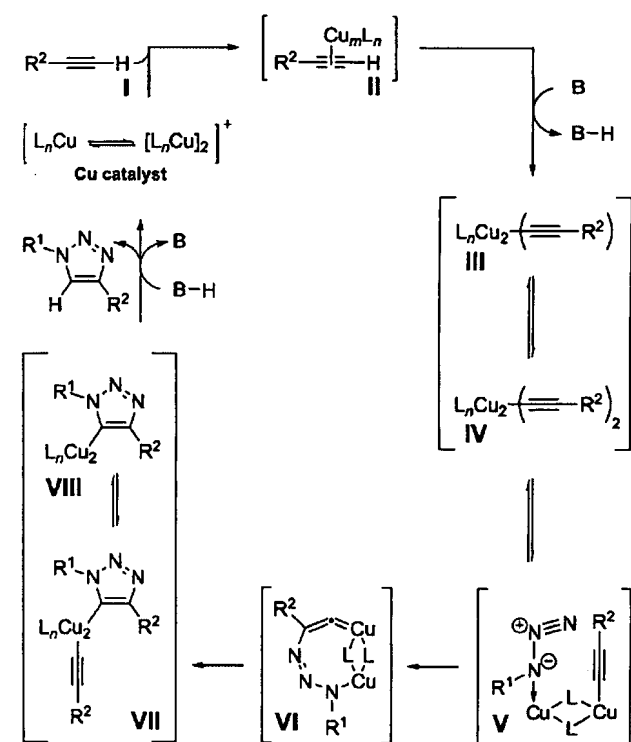
■ *Scheme 5.*

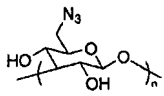*Table 1.* Overview of click reactions with polymers.
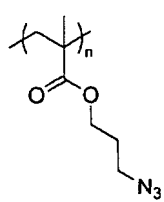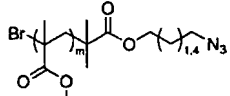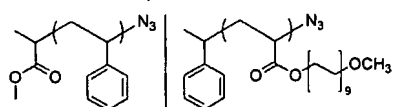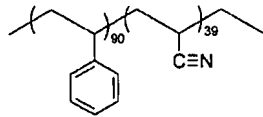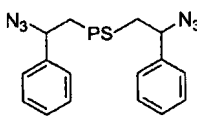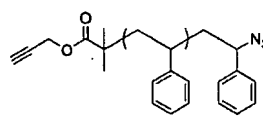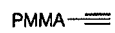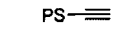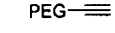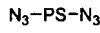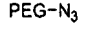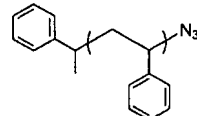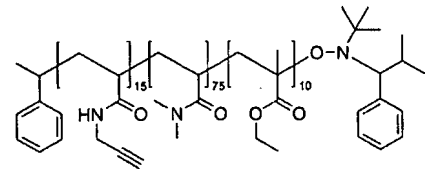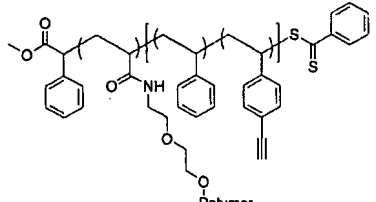
Entry	Polymer/substrate	$\overline{M}_n$	Polymerization method	Catalyst/conditions for 'click' reaction	Ref.
1		18 400	ATRP	$Cu^I Br$/r.t.	[29]
2			ATRP	*N*-alkyl-2-pyridylmethanimine-$Cu^I Br$/70 °C	[30]
3		2 700/6 850	ATRP	$Cu^I Br$/THF/r.t.4, 4'-di-(5-nonyl)-2, 2'-bipyridine	[31]
4a		39 540	ATRP	$NaN_3/ZnCl_2$/120 °C	[28]
4b			ATRP	$NaN_3/ZnCl_2$/120 °C	[28]
5		2 010	ATRP	$Cu^I Br$/PMDETA/r.t.	[32]
6		2 590	ATRP	$Cu^I Br$/DMF/r.t.	[33]
7	PMMA—≡ PS—≡ N₃—PS—N₃ PEG—≡ PEG-N₃		ATRP	$Cu^I I$/DBN/THF/35 °C	[34]
8		4 150	ATRP	CuBr/PMDETA/ THF/35 °C	[35]
9		31 900	nitroxide living-radical polymerization	$CuBr(Ph_3)_3$/DIPEA	[42a,42b]
10		16 200	RAFT	$CuBr(Ph_3)_3$/DIPEA/ THF/H_2O/r.t./3 d	[42b]

Table 1. (Continued)
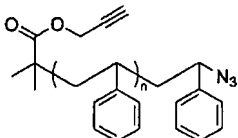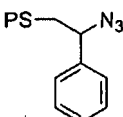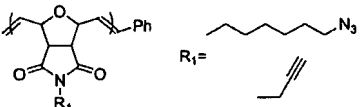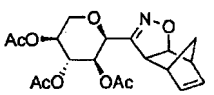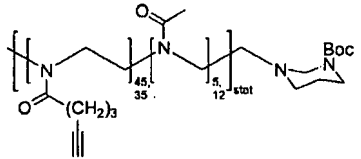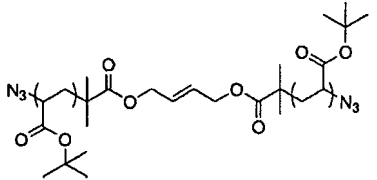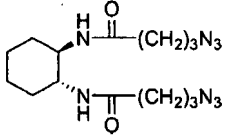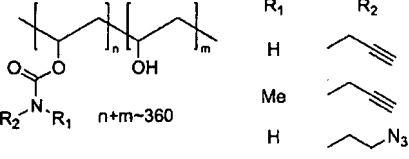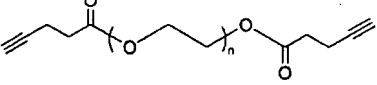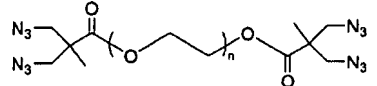
Entry	Polymer/substrate	$\overline{M}_n$	Polymerization method	Catalyst/conditions for 'click' reaction	Ref.
11		4 200	ATRP	Cu^{I}Br/bipyridine/ DMF/120 °C	[37]
12		1 400–18 100	ATRP	Cu(0)/CuBr or Cu^{I}Br/ PMDETA/DMF/r.t.	[36]
13		2 620–10 930	ROMP	Cu^I(Ph$_3$)$_3$Br/DIPEA/ DMF/50 °C	[45]
14		≤33 400	ROMP		[46]
15			living cationic ring-opening polymerization	CuSO$_4$·5H$_2$Owater/ t-BuOH	[49]
16		3 100	quasi-living cationic polymerization of isobutene	Cu^I(Ph$_3$)$_3$Br/DIPEA/ toluene	[48]
17		14 100	ATRP	CuBr/PMDETA/ sodium ascorbate/ DMF	[51]
18				Cu^I/CH$_3$CN	[52]
19		16 000	free-radical polymerization	CuSO$_4$·5H$_2$O/ sodium ascorbate/ H$_2$O/DMSO	[53]
20a		3 400–10 000		CuSO$_4$·5H$_2$O/H$_2$O/t-BuOH = 2:1	[54]
20b					

■ *Table 1.* (Continued)
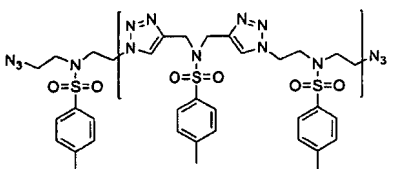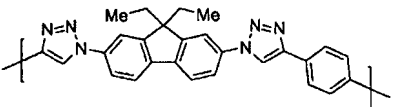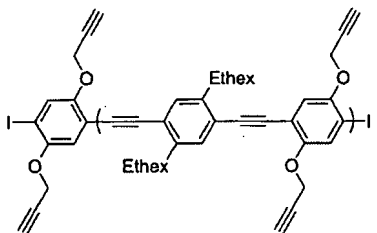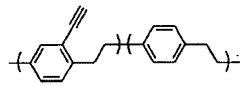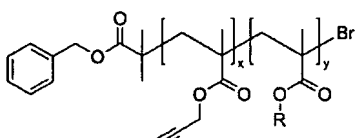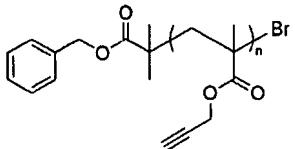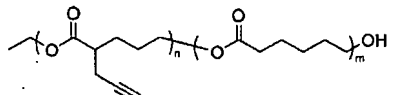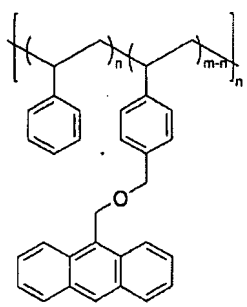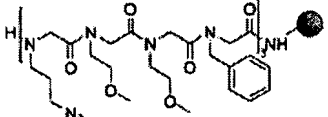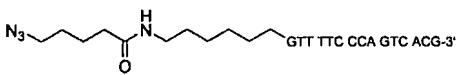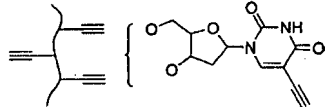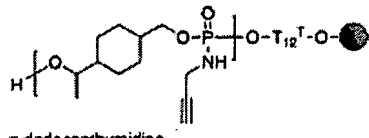
Entry	Polymer/substrate	$\overline{M}_n$	Polymerization method	Catalyst/conditions for 'click' reaction	Ref.
21		12 565	polyaddition	$CuSO_4 \cdot 5H_2O$/sodium ascorbateH_2O/t-BuOH = 1:1/r.t.	[50]
22		<396 000	polyaddition	Cu/Cu(OAc)$_2$/ TBTATHF/CH$_3$CN	[56]
23		24 000	polyaddition	$CuSO_4 \cdot 5H_2O$/ sodium ascorbate	[57]
24			CVD polymerization	$CuSO_4 \cdot 5H_2O$/ sodium ascorbate H_2O/ ^{t}BuOH = 2:1	[58]
25		8 300–17 600	ATRP	Cu(Br)(Ph$_3$)$_3$/DIPEA	[38]
		8 200	ATRP	Cu(Br)(Ph$_3$)$_3$/DIPEA	[38]
26		9 300	anionic ring-opening polymerization	$CuSO_4 \cdot 5H_2O$/ sodium ascorbate/ 100 °C	[59]
27		3 700–27 000	NMP	toluene	[47]
28			sequential stepwise solid-phase synthesis	Cu^I/ascorbic acid/ DIPEA butan-2-ol/ DMF/pyridine	[61]

▌ *Table 1.* (Continued)

Entry	Polymer/substrate	$\overline{M}_n$	Polymerization method	Catalyst/conditions for 'click' reaction	Ref.
29				$Cu^{II}Br_2$/ascorbic acid/propylamine DMSO/r.t.	[62,63]
30	GTT TTC CCA GTC ACG-3'	5 757		$DMSO/H_2O/80\,°C/72h$ (no Cu^I!!)	[65]
31		4 882–5 379	solid-phase synthesis or DNA-polymerase	Cu^I	[66]
32	T_{12}= dodccanthymidine	5 330	solid-phasesynthesis	$CuSO_4 \cdot 5H_2O$/ sodium ascorbate water/methanol	[64]

alkyne,[25,26] to lead to a lowering of the pK_a of the terminal acetylene by up to 9.8 units, thus enabling the attack onto the C–H bond, especially in aqueous systems, has been recently revised[6b] in favor of a binuclear mechanism as shown in Scheme 5.

Support for this hypothesis, which is similar to the monometallic mechanism proposed by Sharpless and co-workers[23,24] by calculation is based on the observation that the rate of catalysis is second order with respect to copper, but with increasing concentration of copper, less reactive species are observed. Overall, this results in a rate acceleration of 10^5 and an absolute regioselectivity of the Cu^I-catalyzed process.[27] Thus both the high regioselectivity and the rate acceleration are accounted for by this mechanism. Similar results are obtained when calculating the Cu^I-catalyzed cycloaddition reaction of azides to nitrile-oxides, for which a similar rate enhancement of the copper-catalyzed reaction in comparison to the purely thermal reaction was observed.

Click Reactions on Linear Polymers and Gels

Since many known polymerization reactions in macromolecular chemistry require the absence of specific functional groups, there is considerable interest in the fixation of ligands onto polymers and gels after a successful polymerization reaction has been conducted. This is most important when living polymerization mechanisms are used, since especially the highly sophisticated chemical mechanism and equilibria of (quasi-)

living polymerization reactions are often highly substrate specific and, therefore, strongly affected by even small amounts of functional groups or the respective coupling agents required for affixation. Another issue concerns the binding of large numbers of ligands onto polymers (i.e., sidechain-modified polymers) or dendrimers, which require highly efficient coupling reactions to this purpose as well. Further interest is directed towards the heterogeneous functionalization of polymers in solvent mixtures. Because of the limited solubility of many polymers, reactants for post-functionalization reactions cannot always be applied in homogeneous solution with the derivatized polymer. In these cases, highly efficient reactions acting in heterogeneous reaction media are desired. Thus it is not surprising that the Sharpless click reaction has been brought into the limelight recently because of its high efficiency, often reaching yields of >99% irrespective of the ligand structure, even in inhomogeneous reaction systems.

Table 1 lists the hitherto known click reactions on various polymers, oligomers, biopolymers, and gels. The nature of the initial polymerization reaction (if known) as well as the structure of the initial, starting polymers are given. Many of the controlled polymerization reactions derive from atom-transfer radical polymerization (ATRP), ring-opening metathesis polymerization (ROMP), and quasi-living cationic polymerization, some are from nitroxide-mediated polymerization (NMP), radical addition-fragmentation transfer (RAFT), or polycondensation reactions. Only a few examples of living anionic polymerization and free radical polymerization reactions have

been described up to now in junction with the click reaction. The catalytic systems used for the click-type reaction are also given, the vast majority with a focus on the copper-catalyzed azide/alkyne click reaction. In general it can be stated that only relatively low-molecular-weight polymers ($\overline{M}_n < 30\,000$ g·mol^{-1}) are used, where a good characterization of the final polymer (in relation to the initial functionalization) is possible. High molecular weights are obtained in only a few cases, where the click reactions are used to build-up polymers with the triazole moiety within the main chain.

Living Radical Polymerizations (ATRP, NMP, and RAFT)

ATRP has been used extensively in conjunction with the azide/alkyne click reaction, and also represent the first, intensely exploited examples of a combination between a (quasi-)living polymerization reaction and the azide/alkyne click chemistry. Three different strategies have been described to combine these two reactions: a) using azido-telechelic macromonomers, b) via alky-telechelic macromonomers, and c) using azido or acetylenic moieties within the sidechain.

A combination between ATRP and an azide/alkyne click reaction has been demonstrated by Matyjaszewski and co-workers[28] using the reaction between pendant nitrile-moieties and sodium azide (Scheme 6). In this variant of the Huisgen 1,3-dipolar cycloaddition reaction, the azide can be fixed onto the sidechains of polyacrylonitrile (PAN, $\overline{M}_n = 39\,450$ g·mol^{-1}) or poly(acrylonitrile-styrene) block copolymers ($\overline{M}_n = 8\,460$ g·mol^{-1}). All polymers were prepared with low polydispersities ($\overline{M}_w/\overline{M}_n < 1.10$) using ATRP methods. The addition of the azide anion is catalyzed by SnCl$_2$ or ZnCl$_2$ to yield the corresponding tetrazole polymers in excellent yields. The addition reaction is reversible, thus liberating nitrogen from the polymers upon heating to temperatures from 120–190 °C.

Sidechain-modified polymers made by an initial ATRP reaction, subsequently followed by an azide/alkyne click reaction were described using 3-azidopropyl methacrylate 1 as the monomer (Scheme 7).[29] This monomer yielded a good ATRP reaction, furnishing polymer 2 in good yields and acceptable polydispersity ($\overline{M}_n = 18\,400$ g·mol^{-1}, $\overline{M}_w/\overline{M}_n = 1.33$). Copolymerization to the block copolymer 3 could be achieved, as well as the click reaction with various terminal acetylenes to give the final polymer 4

in yields higher than 95%, using Cu^{I}Br in N,N-dimethylformamide (DMF) or dimethyl sulfoxide (DMSO) solutions. Despite higher steric hindrance in polymer 2 as compared to the free monomer 1, the click reaction proceeded faster on the polymer, as judged by NMR spectroscopic investigations. Similar to the results obtained with tributyltin acrylate (TBTA),[16a] an anchimeric assistance of the reaction by already formed triazoles was proposed.

End-group modified polymers obtained by an ATRP strategy have been described by several authors.[29–37] An elegant method that places the azido moiety at the initiator part has been demonstrated by Haddleton and co-workers[30] with the initiators 5 (see Scheme 8), acting as initiator for the ATRP of methyl methacrylate via the N-alkyl-2-pyridylmethanimine Cu^{I}Br-initiating system, to yield polymer 6. The initiating efficiency of 5b was found to be higher than those of 5a, presumably because of steric effects. Subsequent click reaction upon addition of terminal alkynes furnished the final polymers 7 in quantitative yields, thus relying on the residual Cu^I catalyst present from the ATRP reaction. A related approach, which relied on the nucleophilic substitution reaction to introduce the azido-moiety after the ATRP process, has been reported by Lutz et al. (see Scheme 9).[31] The polystyrene (PS) polymer ($\overline{M}_n = 2\,700$ g·mol^{-1}; $\overline{M}_w/\overline{M}_n = 1.11$) was prepared by ATRP (Cu^{I}Br/PMDETA), featuring a terminal bromine end-group (8) after the polymerization reaction. Subsequent nucleophilic substitution exchanged the bromine against the azide to furnish the final azido-telechelic polymer 9 by use of sodium azide in DMF as solvent within 3 h at room temperature. The click reaction with various terminal acetylenes (propargylic alcohol, propiolic acid, 2-methylbut-1-en-3-yne) was then conducted on these polymers using the the Cu^{I}Br/4,4'-di-(5-nonyl)-2,2'-bipyridine catalytic system in tetrahydrofuran (THF) as solvent. Again, the reaction proceeded smoothly as judged by NMR spectroscopy to furnish the final polymers 10 in quantitative yields.

A similar strategy that relies on a combination of nucleophilic displacement of the bromine end-group in PS polymers has been achieved by Matyjaszewski and co-workers.[32] As shown in Scheme 10, a bivalent α, ω-dibromo-telechelic PS 11 ($\overline{M}_n = 2\,340$ g·mol^{-1}; $\overline{M}_w/\overline{M}_n = 1.08$) was prepared by ATRP, using 2,6-dibromoheptanedioate as initiator and Cu^{I}Br/PMDETA as the catalytic system. As in Lutz's work,[31] a bromine/azide exchange was conducted to generate the diazido-telechelic polymer 12 quantitatively. Again, click reactions were performed on this bivalent polymer using propargylic alcohol as the acetylenic component. Since two modes of addition processes are possible, gradient polymer elution chromatography (GPEC) was used to separate the
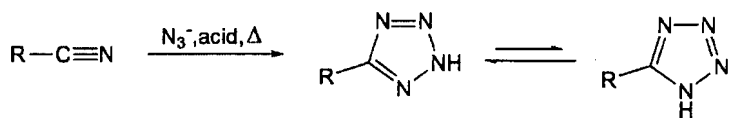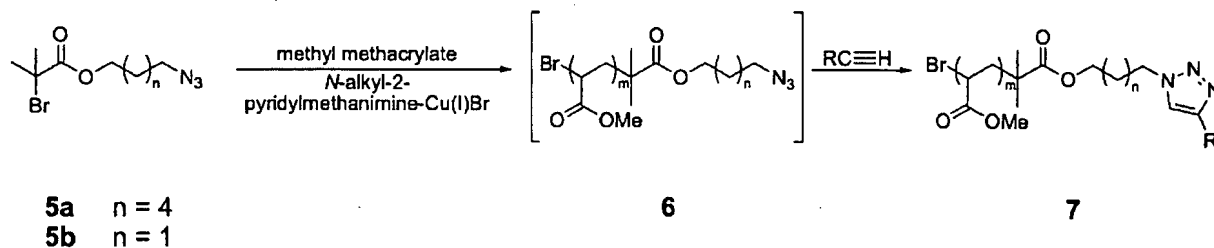

■ Scheme 6.
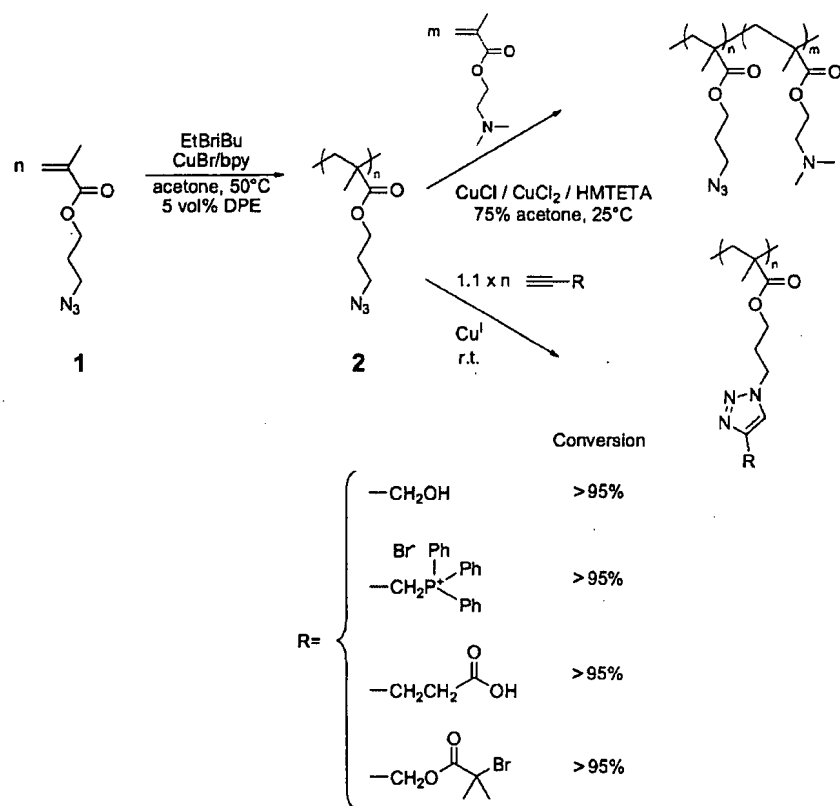
■ *Scheme 7.*

mono- and disubstituted polymers **13** and **14**. This method can separate polymers with similar molecular weight, albeit different polarity by changing the elution-solvent from a poor to a good solvent, which leads to an elution of the chains with the weaker column interactions first. A follow- up of the individual concentrations of the polymers **13** and **14** with time was possible, which led to an individual determination of the apparent rate constants of the click reactions with values of 3.2×10^{-4} and 1.1×10^{-4} s^{-1}, respectively. Therefore, the second click reaction was slower by a factor of 3 than the first one. Obviously, the decreased mobility of the chain overcomes the some-times observed (usually positive) autocatalytic effect of multiple substitution reactions within the azide/alkyne click reactions.

The α-alkyne-ω-azido-terminated PS **16** (prepared by ATRP and subsequent bromine/azide exchange from poly-mer **15**, $\overline{M}_n = 2\,590$ g · mol^{-1}; $\overline{M}_w/\overline{M}_n = 1.11$) formed the platform for a step-growth process on the basis of the click reaction (see Scheme 11).[33] A direct click reaction in the sense of an addition polymerization directly from the ATRP/substitution mixture was accomplished, which generated the Cu^I catalyst by a simple addition of ascorbic acid. After 116 h of reaction time at room temperature, the addition polymer **17** ($\overline{M}_n = 21\,500$ g · mol^{-1}; $\overline{M}_w/\overline{M}_n = 4.85$) was obtained. How-ever, despite this relatively high mole-cular weight, about 18% of unreacted telechelic precursor **16** remained in the reaction mixture. A similar synthetic strategy using the acetylene-telechelic macromonomer **19** in reaction with an α-azido-poly(ethylene glycol) **18** (see Scheme 12) was reported by Opsteen and van Hest.[34] The use of the Cu^{I}I/1,8-diazabicyclo[5.4.0]-undec-7- ene (DBU) catalytic system afforded the poly(methyl methacrylate)-*block*-poly(ethylene glycol) (PMMA-*b*-PEG) diblock copolymer **20** as well as the PMMA-*b*-PS block copolymers in high yields.

The coupling between a peptidic sequence or even a full protein and a telechelic PS-azide to generate bio-hybrid amphiphiles was described by Cornelissen and co-workers[35] (see Scheme 13). The azido-telechelic PS **21** ($\overline{M}_n = 4\,150$ g · mol^{-1}; $\overline{M}_w/\overline{M}_n = 1.15$) was directly coupled to the 3-butynyl-linked peptide **22**, which carried a coumarin dye as fluorophore. The coupling-reaction was achieved with Cu^{I}Br/PMDETA in THF as solvent. Proof of the efficiency of the coupling reaction in the resulting biohybrid amphiphile **23** was achieved by matrix- assisted laser desorption-ionization (MALDI) mass spectrometry, which clearly demonstrated the shift in molecular weight upon addition of the peptidic sequence. Surprisingly,

■ *Scheme 8.*
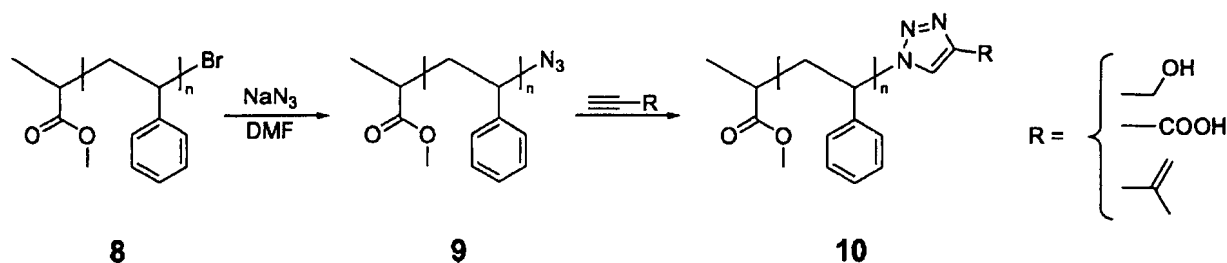
■ *Scheme 9.*

whereas the conventional aqueous $Cu^{II}SO_4$/ascorbate catalytic system failed with substrates **21** and **22** in THF/water mixtures, the conjugation of **21** to appropriately functionalized BSA (bovine serum albumin) was successful.

The syntheses of star polymers by ATRP/click methodology has been described by Gao and Matyjaszewski starting from telechelic azido-PS polymers (see Scheme 14).[36] A series of PSs **24** (molecular weights ranging from $\overline{M}_n = 1400$ to $18\,100\ g \cdot mol^{-1}$) was reacted with the bi-, tri-, and tetravalent alkynes **25**, **26**, and **27** respectively, to furnish the final star-type polymers **28**, **29**, and **30**. A small but significant influence of added Cu^0 species was found with respect to the reaction yield and efficiency, which led to an improvement of the reaction upon addition of small quantities of Cu^0 species. However, reaction efficiencies decreased with increasing molecular weights, which ranged from 95 ($\overline{M}_n = 1400\ g \cdot mol^{-1}$) to 80% ($\overline{M}_n = 18\,100\ g \cdot mol^{-1}$). The method represents a highly universal approach towards star-type polymers with various polymeric sequences and structures.

The formation of macrocyclic polymeric rings has been demonstrated by Laurent and Grayson using the telechelic polymer **31** (see Scheme 15).[37] As known from organic synthesis, large rings are obtained with only some selected macrocyclization reactions, mostly under high-dilution methods. Since the azide/alkyne click reaction is a highly efficient reaction that works under moderate reaction conditions and, most importantly, involves a template effect by the Cu intermediate, a cyclization approach is highly interesting. Thus the starting PS polymer bearing terminal acetylenic and bromine moieties **16** (prepared by ATRP, $\overline{M}_n = 4200\ g \cdot mol^{-1}$; $\overline{M}_w/\overline{M}_n = 1.06$) was transformed into its azido-analogue **31**. Despite the use of the

ultrahigh dilution conditions usually required for these types of macrocyclization reactions, a continuous addition of the substrate by a syringe pump to a solution that contained the Cu^IBr/bipyridine catalyst in DMF (0.1×10^{-3} M) was performed. The successful cyclization to yield **32** was accomplished as proven by NMR measurements (**32**: $\overline{M}_n = 4170\ g \cdot mol^{-1}$) upon inspection of the triazole resonances at 4.3–4.8 and 5.3–5.7 ppm, respectively. This demonstrates the high efficiency of the click reaction for the future preparation of macro(polymeric)cycles by a simple ring-closure reaction.

The attachment of carbohydrates to sidechain-modified polymethylacrylates has been demonstrated by Haddleton and co-workers (see Scheme 16).[38] Starting from the sidechain-modified PMMA **33**, which bears multiple acetylenic moieties, a number of carbohydrates can be affixed (e.g., 1-azidosugars, 6-azidosugars). In accordance with previous investigations on carbohydrates[39,40] and cyclodextrins[41] the carbohydrates can be either fully protected or, alternatively, can be used in the unprotected state with $(Ph_3)_3Cu^IBr$ and diisopropylethylamine (DIPEA) in DMSO as the catalytic system. As an elegant application, the generation of a library of mannose- and galactose-containing polymers **35** and **36** by a 'co-clicking' approach was reported. The relative amounts of the two different carbohydrates were varied and the reaction checked for the selective mannose/concanavalin A interaction in relation to the achieved ligand density. Significant effects between the ligand densities and the binding efficiencies were observed.

Recently there have been a few examples for the combination of NMP and the azide/alkyne click reaction (Scheme 17).[42] Various copolymers (e.g., the water-soluble terpolymer **37**) were prepared by NMP to enable the direct
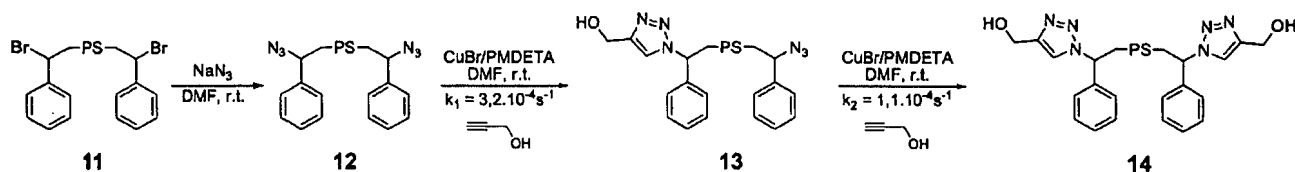
■ *Scheme 10.*
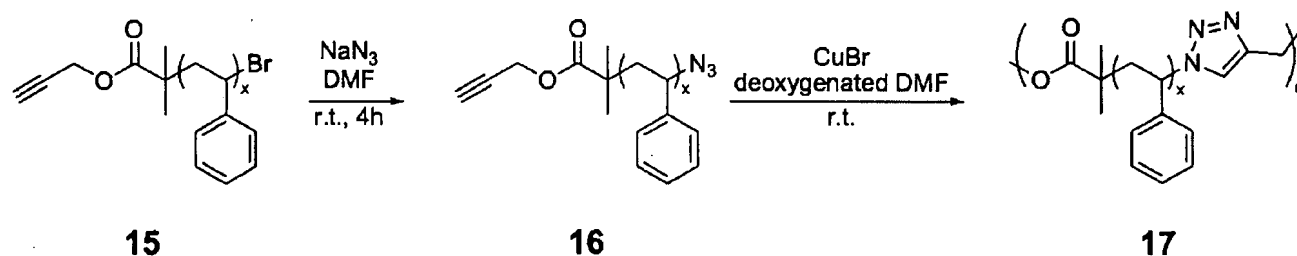
Scheme 11.

introduction of the terminal acetylenic moieties after deprotection with tetrabutylammonium fluoride (TBAF). Subsequent attachment of azido-moieties (38) was effected using $(Ph_3)_3Cu^IBr$ and DIPEA to furnish a large variety of different polymers 40 in high yields ($\approx$98% coupling efficiency) as judged by HPLC and NMR methods. A combination of RAFT and the azide/alkyne click reaction has been reported,[42b] taking advantage of the RAFT polymerization process, since the corresponding NMP process did not succeed in maintaining the corresponding terminal acetylenic moieties. However, even in the RAFT process, protection of the terminal acetylene moiety as a trimethylsilyl derivative was required. The final click reaction furnished the corresponding coumarin derivative within a block-copolymeric micelle.

A polycondensation approach was first reported by Krasla and Steinke[43] using bivalent azides and alkynes as building blocks for polymers (Scheme 18). The 1,3-dipolar cycloaddition process between the acetylene 41 and the azide 42 in this case was catalyzed by cucurbituril according to a previous report by Mock et al.,[44] who demonstrated the efficiency of this catalytic system in the synthesis of polyrotaxanes. The cucurbituril in this case acts as a bridge between the alkyne and the acetylene, thus easing the linkage forming the 1,3-dipolar cycloaddition process by a preorganized structure to yield the rotaxane 43 as well as the polymeric rotaxane 44.

ROMP, Cationic Polymerization, and Anionic Polymerization

Few examples have been reported that combine ROMP and (quasi-)living cationic polymerization with click reactions.

Thus Binder et al.[45] (Scheme 19) have developed efficient attachment strategies of supramolecular receptors (hydrogen-bonding structures) onto poly(oxynorbornenes).[45a] Thus either norbornene monomers (45, 46) as well as sidechain-derivatized polymers (47) bearing azido or acetylenic moieties can be used to effect efficient linking strategies to yield the final derivatized polymers 48. The critical point in the combination of poly(oxynorbornene) chemistry and click chemistry lies in the control and thus suppression of the concurring thermal addition of azides onto the norbornene double bond. Thus only oxynorbornene monomers can be used, because of their reduced ring strain with respect to norbornenes and thus reactivity in the non-catalyzed process. By developing the concept either from the monomer or the post-modification of the final polymers, a large variety of homo[45a,45b] and block copolymers[45d,45e] can be prepared using only a small set of starting monomers. Advantageous in this respect is the possibility to generate polymeric libraries from only a small set of precursor polymers. Another strategy using ROMP in conjunction with Diels-Alder-type cycloaddition reactions has been described by Paton and co-workers,[46] which relies on nitriloxide addition onto the norbornene double bond. This highly efficient reaction runs in the absence of copper or other metal salts at moderate conditions, which enables the attachment of various carbohydrates onto the poly(norbornene) backbone. A conventional anthracene/maleimide click reaction that relies on a highly efficient, purely thermal Diels-Alder reaction has been recently reported by several authors.[47]

Two examples for the combination of cationic polymerization reactions with click reactions have been described. One example describes the fixation of supramolecular ligands onto mono-, bi-, and trivalent telechelic
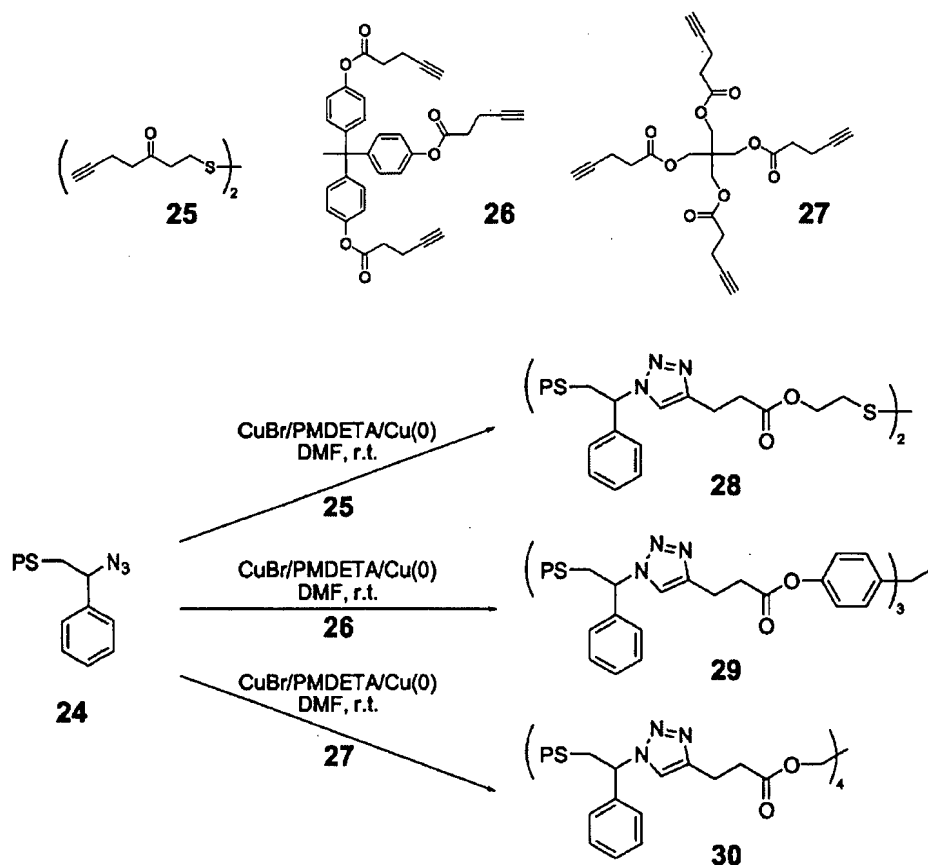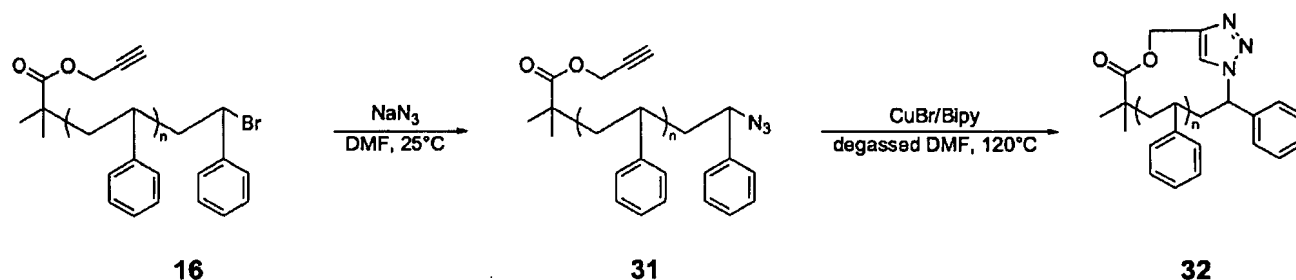

Scheme 12.

■ *Scheme 13.*

Cu[I]Br as the catalyst, and lead to yields above 94% (Scheme 20). Thus the reaction works satisfyingly at the interface, which enables the attachment of water-soluble substrates onto water-insoluble polymers, such as the trivalent polyisobutylene **49**, to result in a large variety of trivalently substituted polyisobutylenes **50**. Another example of cationic polymerization has been demonstrated by the living cationic polymerization of poly(1, 3- oxazolines) using 2-(pent-4-ynyl)-2-oxazoline as the monomer.[49a] The attachment of some functional groups onto the sidechain-functionalized poly(-oxazoline) was demonstrated by MALDI, which revealed the high fidelity of this derivatization reaction. A recent example of the combination of anionic polymerization with click reactions has been reported.[49b] 1-Ethyl-4- hydroxybenzene has been used as a monomer in several block copolymers (e.g., PMMA, PS) and subsequently transformed into the corresponding oxypropynes by classical Williams or Mitsunobu strategies. The click reactions could then be performed on these substrates.

polyisobutylenes, prepared by quasi-living cationic polymerization $(\overline{M}_n = 3\,100$ g·mol^{-1}; $\overline{M}_w/\overline{M}_n = 1.10)$.[48] The reaction has been performed in biphasic reaction systems, which feature toluene/water solvent mixtures and

■ *Scheme 14.*

■ *Scheme 15.*

R = CH₃ **33**
R = mPEG₃₀₀ **34**

R = CH₃ **35**
R = mPEG₃₀₀ **36**

■ *Scheme 16.*

37

38 CuBr(PPh₃)₃ | DIPEA **39**

40
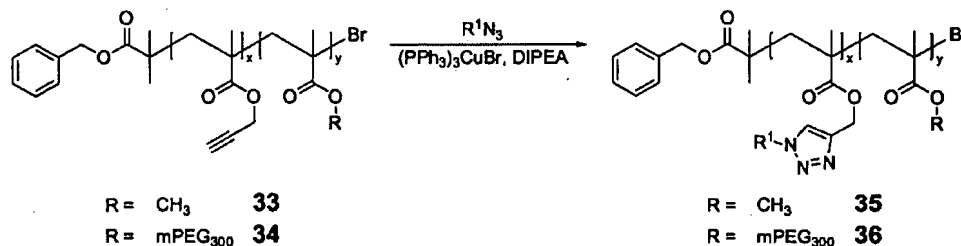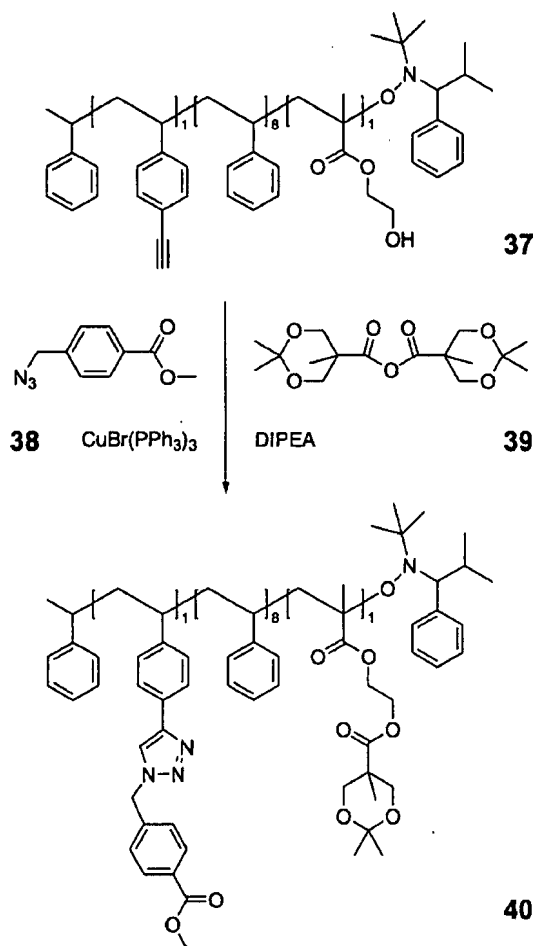
■ *Scheme 17.*

Gels and Networks

Certainly, the click reaction opens an interesting prospect for crosslinking reactions, to furnish gels and networks as the resulting materials. A low temperature, combined with a high efficiency, may satisfy the mild reaction conditions required for gel-formation or templating processes.

The first contribution for the in-situ polymerization of azides and alkynes into networks has been contributed by Sharpless and co-workers.[50] Two approaches were conducted to generate polymeric structures: bivalent azides and bivalent acetylenes were polymerized using $Cu^{II}SO_4$ and sodium ascorbate in a t-butyl alcohol/water mixture to furnish polycondensates with molecular weights below 12 500 g · mol^{-1}. This approach was taken as a starting point to prepare resin-type structures derived from bi- and trivalent azides and alkynes (Scheme 21). A large variety of trivalent acetylenes as well as trivalent azides were used in this condensation approach to yield a crosslinked resin material. The crosslinking process was used to link metallic structures (i.e., copper plates) by non-covalent interactions exerted by the acetylenes and formed triazoles, respectively. This method provides a new way to glue planar metallic plates by an efficient crosslinking process reminiscent of epoxy resins.

Another type of crosslinking reaction has been described by Turro and co-workers.[51] Bivalent, α,ω-diazido-telechelic poly(t-butyl acrylates) **51** ($\overline{M}_n = 14\,100$ g · mol^{-1}; $\overline{M}_w/\overline{M}_n = 1.12$), prepared by ATRP methods, were crosslinked into gels **52** by reaction with tri- and tetravalent acetylenes, such as **53** (see Scheme 22).
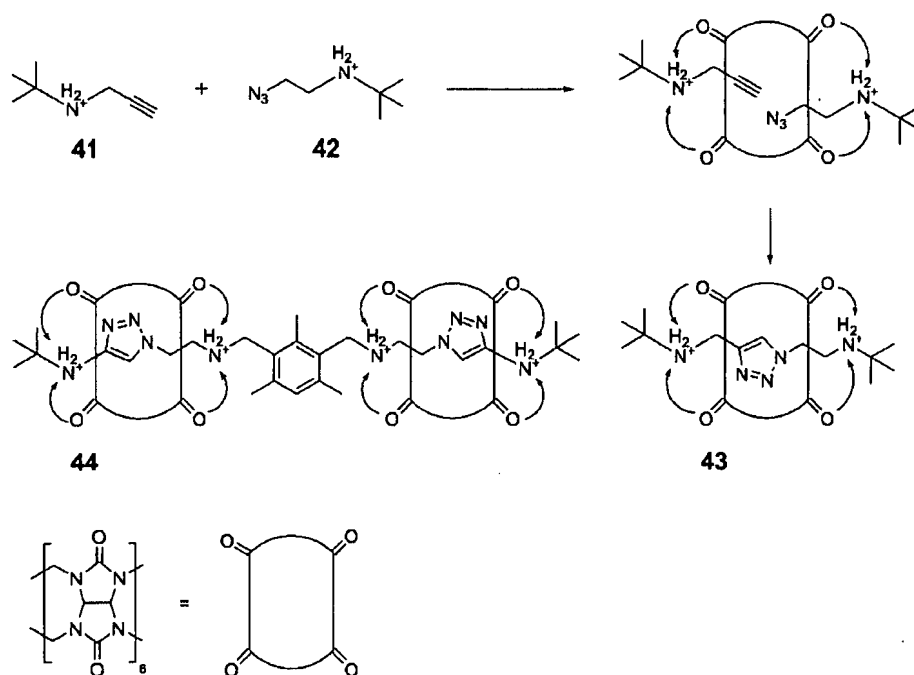
■ *Scheme 18.*

The optimal (and fastest) reaction conditions were found with a reaction system that consisted of Cu^{I}Br/PMDETA with sodium ascorbate in DMF as solvent, and required ≈5 min for a complete crosslinking reaction. The fidelity of the crosslinking reaction was proven by ozonolysis of the central double bond in polymer **52**, which enabled control of the destroyed polymer networks by gel permeation chromatography (GPC) analysis as a result of a significant molecular weight shift to $\overline{M}_n = 7\,300$ g · mol^{-1} with $\overline{M}_w/\overline{M}_n = 1.21$. Since hardly any initially present polymer **51** was detected, the crosslinking reaction was said to be complete, and reflected a direct transformation of the theoretical crosslinking density into the actual one. The high efficiency of the crosslinking reaction also enables the crosslinking of highly preorganized, supramolecular systems. Finn and co-workers[52] report a process wherein a network of hydrogen-bonding systems forms fibers, and subsequently organogels (see Scheme 23). Terminal acetylenic groups were affixed to the hydrophobic end-groups of the ordered system **54**, and subsequently crosslinked by a short, bivalent α,ω-diazide, to yield the crosslinked gel **55**. Each reaction was performed in acetonitrile, with a gelator concentration of 3% and a gelator/crosslinker ratio of 10:1, using Cu^{I}I as the catalyst system. Several features are important in the final, crosslinked products: a) the structure of the fibers initially present in **54** are fully transformed into a fiber structure in the crosslinked structure **55**, b) the crosslinked gels **55** retain their stability up to temperatures even above the

boiling point of acetonitrile, and c) the storage moduli G′ increased significantly during crosslinking, which clearly demonstrated the formation of a rigid gel from the previously dynamic organogel **54**.

Crosslinking of statistically substituted poly(vinyl alcohol)s has been recently described by Ossipov and Hilborn.[53] Two differently substituted poly(vinyl alcohols), derivatized by carbamate linkages to give polymer **56** and **57**, respectively (see Scheme 24) were prepared. Crosslinking was achieved using the Cu^{II}SO$_4$/sodium ascorbate coupling reaction in aqueous systems. Upon systematically varying the amount of Cu^{II} in solution, a minimum of 20 mol-% with respect to the functional groups concentration was detected, which formed hydrogels of the general structure **58**. This hints at incomplete crosslinking reactions at lower Cu^{II} concentrations, and thus at a minimum amount of Cu in order to effect gel formation. A similar approach, albeit with different monomeric and oligomeric components has been described by Hawker and co-workers.[54] As shown in Scheme 25, a telechelic poly(ethylene oxide) **59** ($\overline{M}_n = 3\,400$–$10\,000$ g · mol^{-1}) was crosslinked with a tetravalent azide **60**, to yield crosslinked hydrogels **61**. Compared to gels with similar structure, crosslinked via photochemical activation, the click crosslinked gels display strongly enhanced tensile stress and strain stabilities. The reasoning states that in contrast to radically crosslinked gels (where often dense clusters are surrounded by a weakly crosslinked matrix), perfectly crosslinked structures are obtained by the click
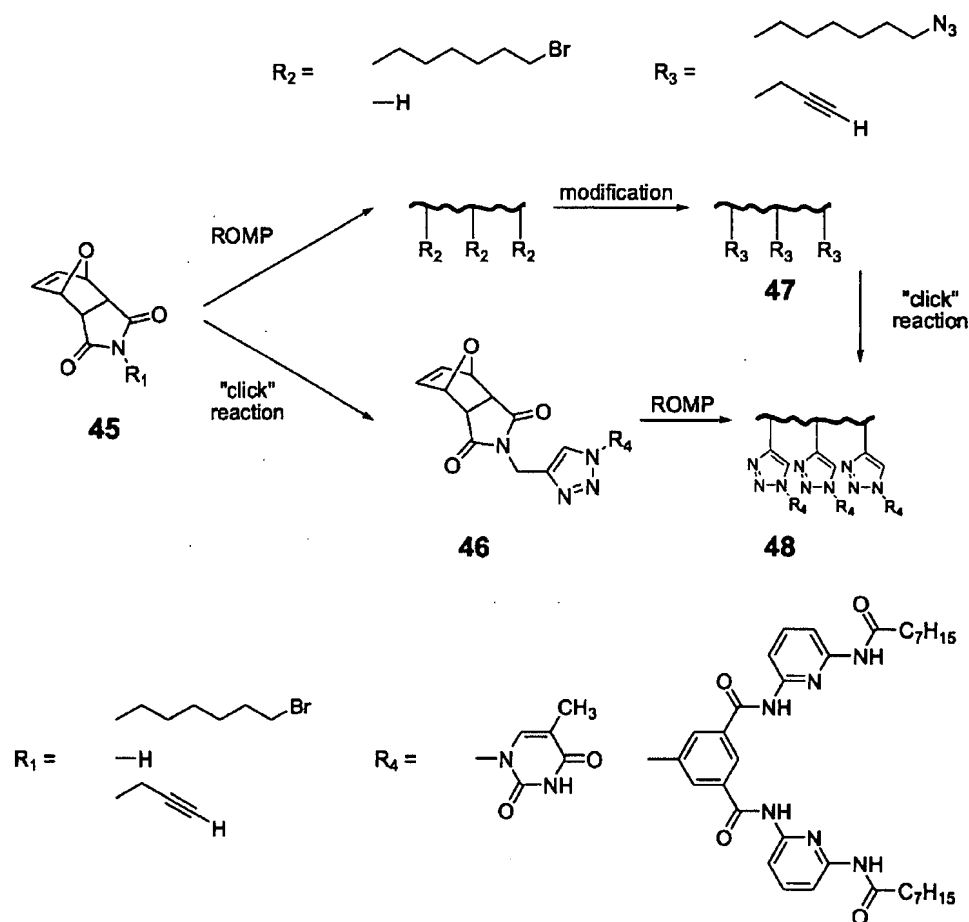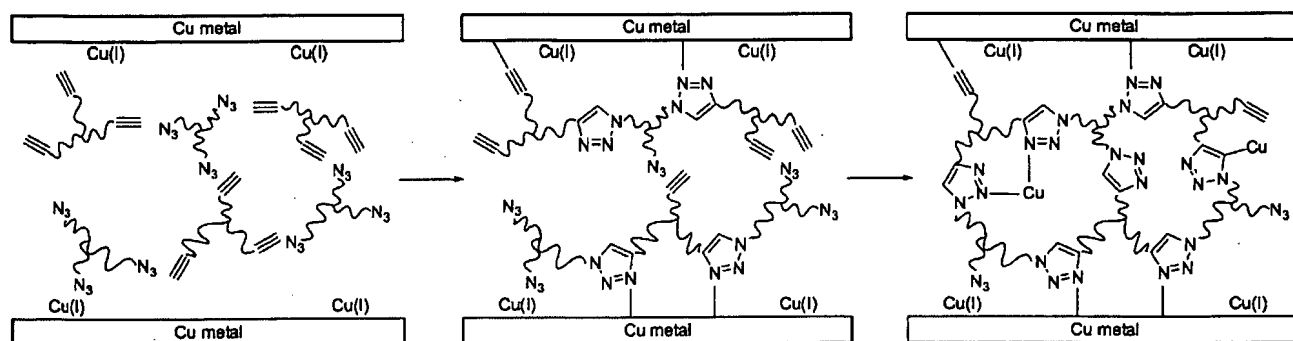
Scheme 19.
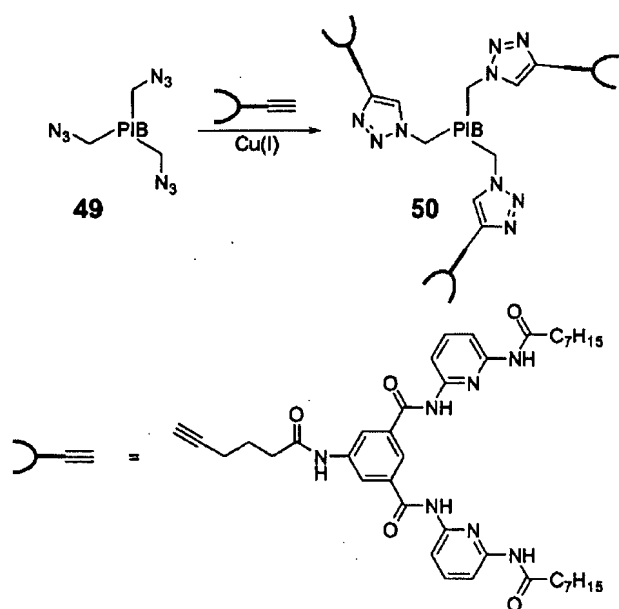
Scheme 20.

reactions, since the perfection is high because of the high efficiency of the crosslinking reaction.

This high dimensional stability of the gels that result from the azide/alkyne click reaction has prompted the generation of molecularly imprinted reactors for the 1,3-dipolar cycloaddition reaction as reported by Mosbach and co-workers.[55] Catalytic effects with respect to the regioselectivity of the cycloaddition reaction were observed.

Click Reactions on Other Polymers

The click reaction has been used in a variety of other polymerization reactions not related to living of quasi-living polymerizations. Thus a new strategy aimed at the synthesis of new conjugated polymers based on poly(fluorene)s was developed (see Scheme 26).[56] Starting from a 4,4′-disubstituted fluorine building block **62** and another 4,4′-diacetylenic fluorene **63**, a click reaction in the sense of a polyaddition process was conducted. Finally

■ *Scheme 21.*

the polymers **64**, with molecular weights up to $\overline{M}_n = $ 13 000 g·mol^{-1} with a $\overline{M}_w/\overline{M}_n = 1.61$ were obtained. However, because of the presence of the triazole moieties, a poor electronic communication between the individual π-systems was observed, which resulted in a poor conductivity. Bunz and co-workers[57] have demonstrated the importance of the click reaction for the construction of poly(p-phenyleneethynylene)s (see Scheme 27).

Sidechain-modified poly(phenyleneethynylene)s **65** that bear terminal silyl-acetylenic moieties were subjected to an initial deprotection to furnish the free acetylenes, and were subsequently subjected to a click reaction to yield the substituted polymers **66**. This enabled the introduction of a large variety of sidechain modifications into these polymers, leaving the main-chain acetylenic bond entirely unaffected. A related reaction that relied on terminal acetylenes in poly[(4-ethynyl-p-xylylene)-co-(p-xylylene)] is shown in Scheme 28.[58] The polymer was prepared as a thin film by chemical vapor deposition (CVD) of ethynyl[2,2]paracyclophane, which resulted in a film thickness of ≈50 nm. Subsequently, a layer of

biotinazide as well as sodium ascorbate was applied to the film, which resulted in an even distribution of the two components inside the polymer matrix. Conventional microcontact printing supplied Cu(SO$_4$) to specific regions of the polymer film to result in a local click reaction between the biotin and the sidechain acetylenic components. The binding of biotin was monitored by a labeled streptavidin/dye conjugate, which proved the generation of patterns in the 50 μm range. This example demonstrates the fine applicability of this reaction onto polymer films by a direct printing approach.

Other sidechain modifications of polymers[59] have been reported using azido- or acetylene-functionalized polymers or oligomers. An example for an aliphatic co-polyester with pendent acetylenic groups has been described by Emrick and co-workers[59a] and Jerome[59b,59c] (Scheme 29). Thus the co-polyester **67** (prepared by Sn(OTf)$_2$-mediated polymerization of the respective lactones), was reacted with oligomeric, α-azido(ethylene glycols) **68** to furnish the final grafted polymers **69**. In addition, small peptidic sequences that consisted of hexynoylamido-functionalized RGDS
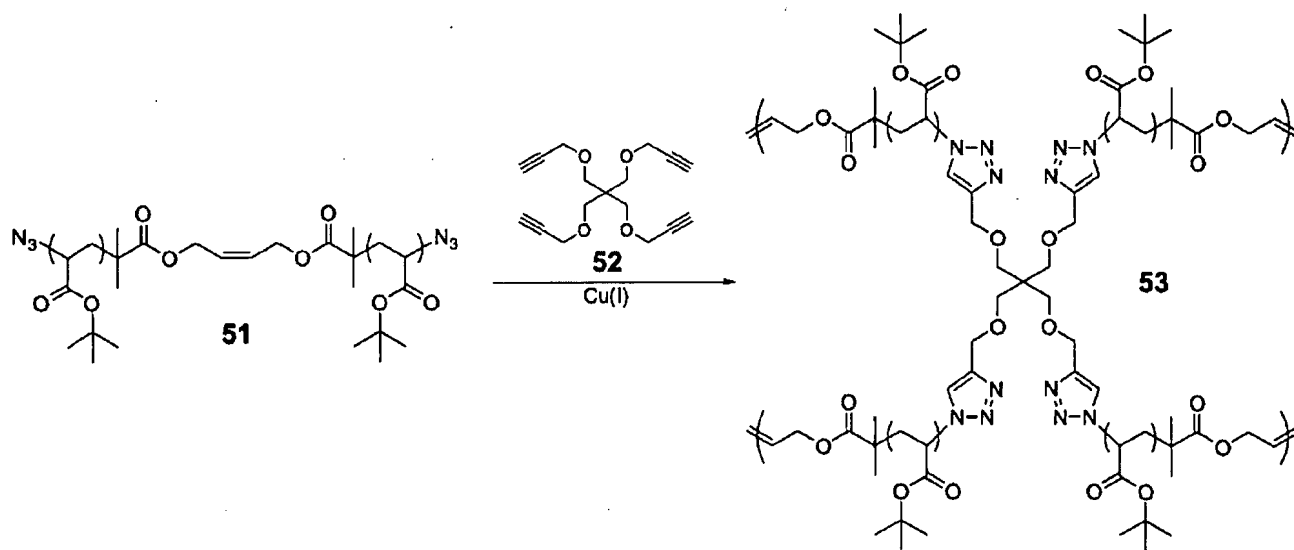
■ *Scheme 22.*
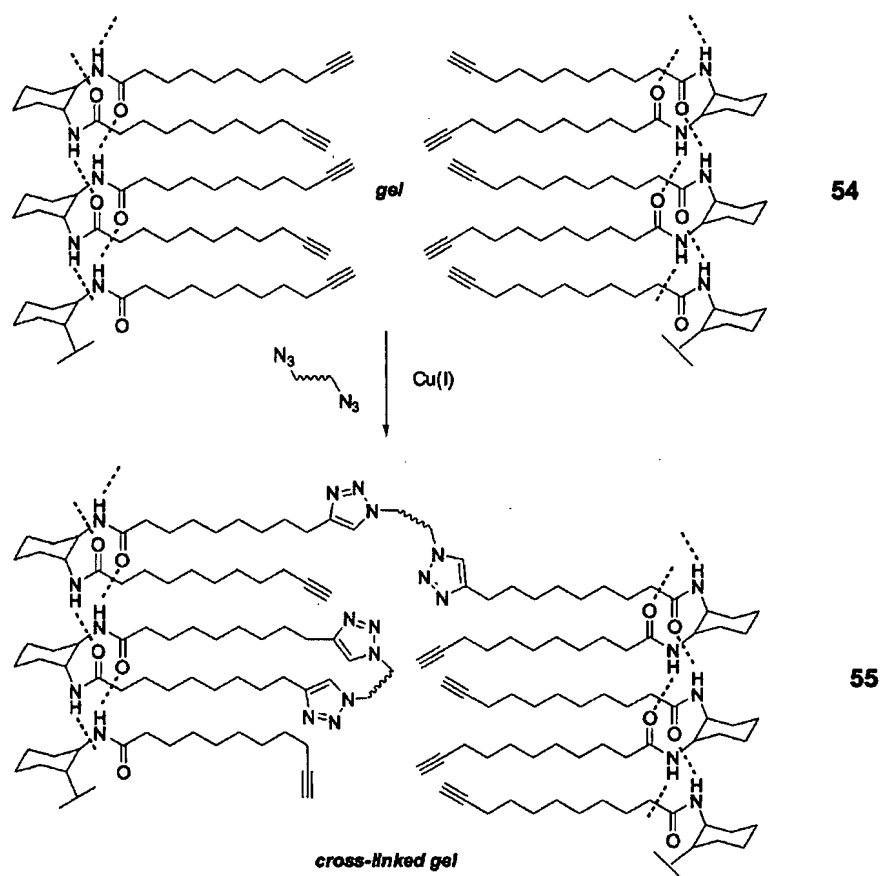
■ Scheme 23.

(Arg-Gly-Asp-Ser)sequences were successfully reacted, to yield biocompatible polymers for tissue engineering purposes.

There are many examples of coupling reactions onto biomolecules, in particular proteins, nucleic acids, as well as whole-cell systems. Furthermore, affinity based protein profiling (ABPP), as well as the application of the click reaction has been used in combinatorial chemistry. [8,9,60] Since a full description of these issues would be beyond the scope of this article, the reader is referred to recent reviews in the area. In this article, only defined oligomeric structures will be discussed.

Reverse reaction onto synthetically prepared peptides that bear sidechain-modified acetylenic sequences was reported by Kirschbaum and co-workers.[61] In this work, defined sequences of oligopeptides were generated by a stepwise synthetic approach, including pendent azido groups of acetylenic moieties. Subsequent azide/alkyne click reactions under copper catalysis furnished the final, sidechain-modified oligopeptides in high yield.

Polysaccharides have been used recently as scaffolds for the azide/alkyne click reaction as reported by Shinkai and co-workers[62] (see Scheme 30). 6-Azido-6-deoxycurdlan **70** was prepared in a short reaction sequence, featuring

bromination and subsequent azide exchange. A large variety of different substrates **71a–71e**, bearing terminal acetylenes, was then coupled by use of $Cu^{II}Br_2$/ascorbic acid/propylamine as a catalytic system to yield **72**. Both ammonia as well as propylamine were found to strongly accelerate the coupling reaction, which reached conversions of the azide after $\approx$1 h. Another example reporting the synthesis of pseudo-oligosaccharides, which relied on triazole linkages, has been reported recently.[63]

Several examples for click chemistry on oligonucleotides were reported.[64–67] The purely thermal 2,3-thermal Huisgen reaction was used by Ju and co-workers[65] to link a rhodamine dye to the end of oligonucleotides. The starting azido-tagged oligonucleotide was subjected to the thermal dipolar cycloaddition process at 80 °C with an acetylene-modified rhodamine dye. The reaction was not highly regioselective and both the 1,3- and 1,4-regio-isomers of the dye-labeled oligonucleotide were obtained. This method, if conducted under action of Cu^I, may definitely represent a low-temperature variant of the reported thermal labeling procedure. A highly versatile approach was reported by Carell and co-workers.[66] As shown in Scheme 31, an alkyne-sidechain-modified 16-mer DNA **73** was prepared and subjected to azide/
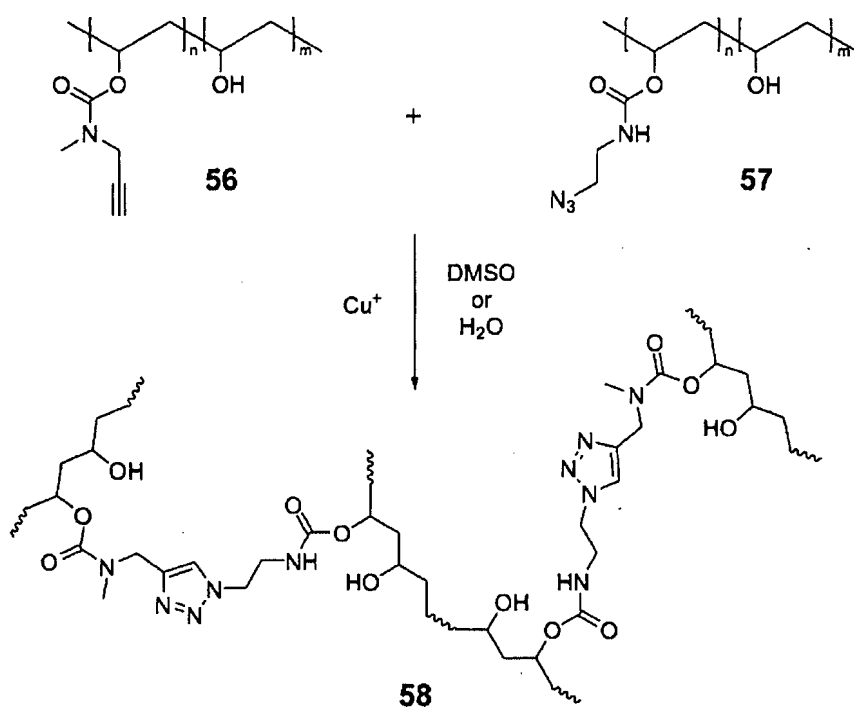
Scheme 24.
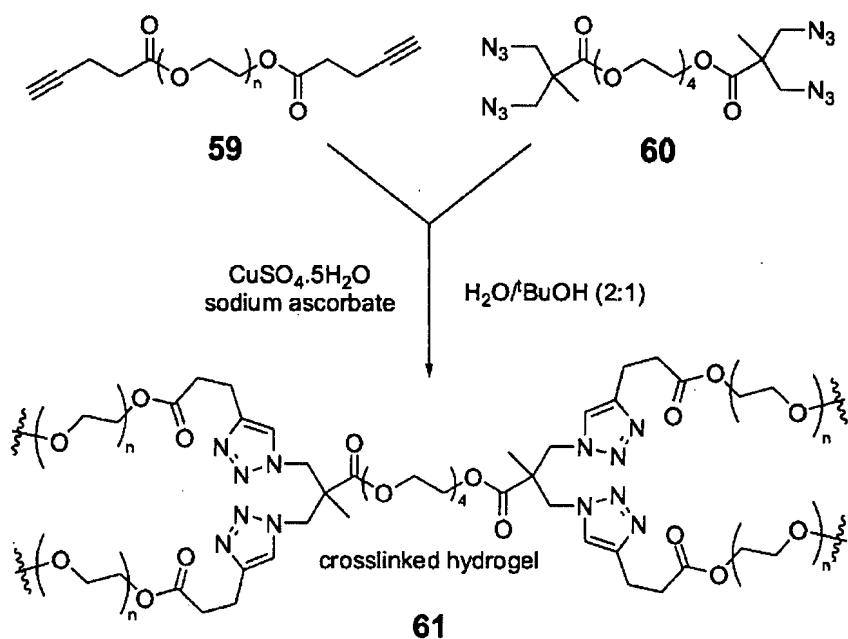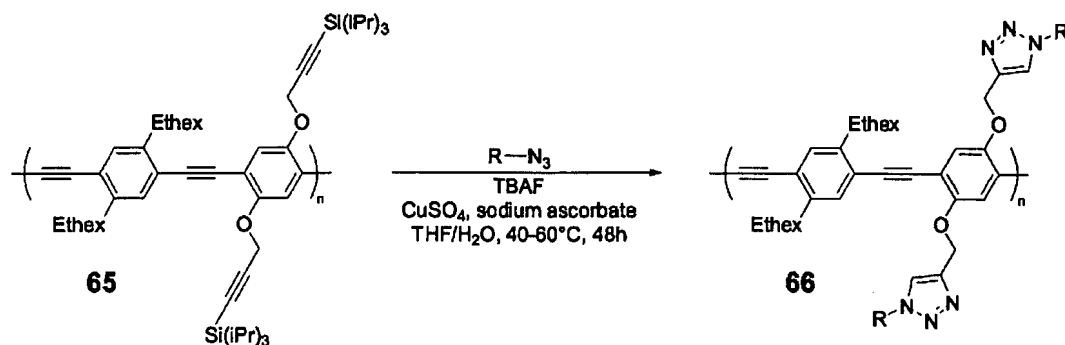

Scheme 25.
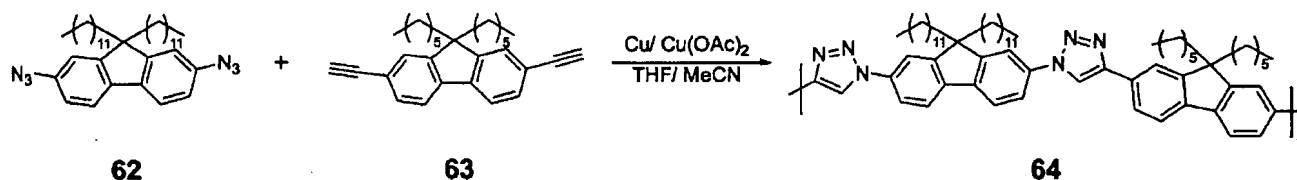
Scheme 26.

alkyne click reactions to yield the modified DNA **74**. The synthesis of **73** relied on the incorporation of the unnatural nucleobases **75** and **76** into the DNA-strand by chemical synthesis on the solid phase. Subsequently, dyes and/or carbohydrates could be bound to the DNA using the classical Cu[I]/TBTA catalytic system. Furthermore, dendrites could be added to these DNA sequences, enabling the binding of metal salts and the subsequent metallization of the DNA by Ag deposition.[67]

Rotaxanes, p-Cyclophanes and Calixarenes

The linking of large molecular fragments is an important synthetic challenge in supramolecular chemistry to generate, e.g., cyclic structures and interlocked molecules. Therefore, the click reaction on larger organic molecules touches upon classical polymer chemistry and is included in this review. Ryu and Zhao[41a] have studied the click reaction on calixarenes modified with five terminal azido moieties. Using the Cu[II]SO$_4$/sodium ascorbate system, D-gluconamide residues were bound to the lower rim of the calixarene, thus providing extremely simple access to functionalized calix[4]arenes. A similar approach has been undertaken by Santoyo-Gonzales and co-workers[41b] to generate multivalent neoglycoconjugates by the addition of 1-O-propargyl-gylosides onto 1-O-(β-azidoethyl)-glycosides. The addition onto multivalent substrates (e.g., cyclodextrins) has also been reported.[41b]

The formation of [2]-, [3]- and [4]-rotaxanes is another example for the high efficiency of the click reaction.

Starting from a previous discovery[68,69] that either preorganizational effects as well as the cationic nature of cucurbituril can effect an efficient azide/alkyne click process, Tuncel and Steinke[70] have prepared rotaxanes wherein the cucurbituril moiety serves either as catalyst as well as a rotaxane-part during an azide/alkyne click reaction. Higher rotaxanes are also accessible using bifunctional building blocks as well as under generation of rotaxanes with a pH-responsive moiety.[71] Another example of supramolecular compounds has been reported by Stoddart and co-workers.[72] The critical step in the formation of interlocked structures is the fixation of the intermediate supramolecular complexes. Thus, usually after self assembly, large 'stoppers' are applied to the end-groups, which allow the final locked systems to fix the interlocked bundles. A simple approach using 1,3-dipolar cycloaddition chemistry was developed. The 'locking-in' of the complex was accomplished by a (thermal) 1,3-dipolar cycloaddition reaction using di-tert-butylacetylenedicarboxylate at 40 °C, and then furnishing the final rotaxane. This reaction is faster than the conventional thermal Huisgen process because of the preorganization of the ligands. Thus this reaction can be performed in preorganized samples without Cu[I] catalysis with good efficiency. A recent similar example of Sauvage and co-workers[73] uses a preorganizational stoppering method that relies on metal complexation. Despite the fact that this method will be only applicable to a limited number of structurally diverse rotaxanes, the mechanism demonstrates the efficiency of this linkage reaction.
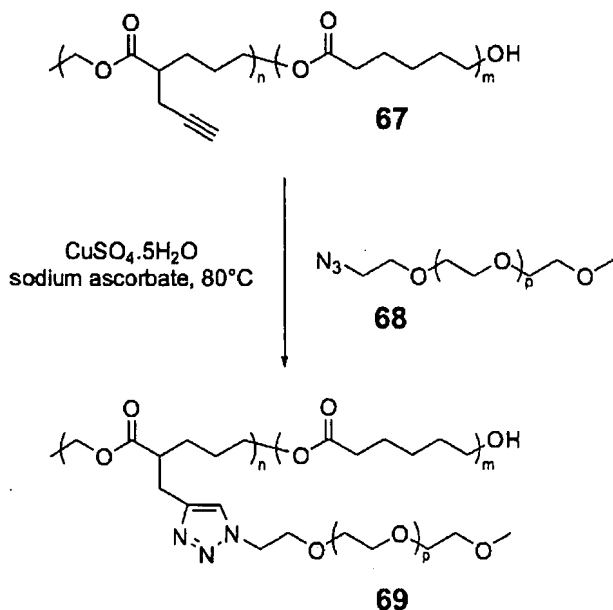

Scheme 27.
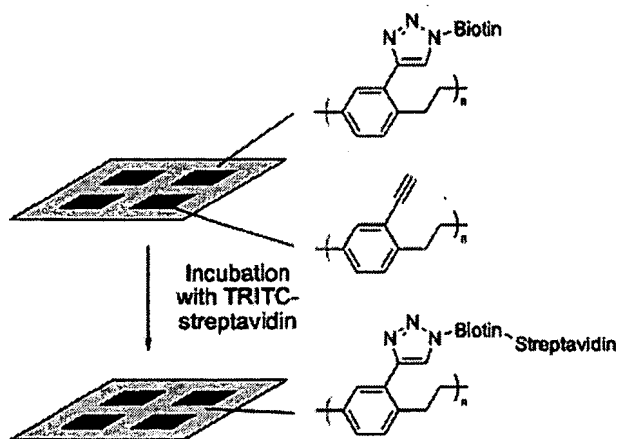
■ Scheme 28.

Click Reactions on Dendrimers

The literature on applications of the azide/alkyne click reaction for the modification of dendrimers has expanded considerably during the past two years. Because of the high yields promoted by click chemistry, the application on multiple reaction sites can be easily derived. Thus in order to fully substitute a generation-3 dendrimer with 96 chain ends using a reaction with 99% yield, only 38% of the fully substituted product can be obtained. Thus dendrimeric systems are an important field of investigation, since the click reaction is not only a very high yielding reaction, insensitive to most functional groups present in the reaction partners, but also allows reaction in sterically hindered environments. Thus the fixation of dendrons onto polymeric backbones, the synthesis of dendrimers

and hyperbranched polymers, and finally the derivatization of the dendrimer's surface is an important field for click reactions.

The surface functionalization of dendrimers derived from 3, 5-dihydroxybenzoic acid using a large variety of surface-bound peptides has been achieved.[74] As shown in Scheme 32, starting from a dendrimer with a surface bearing multiple acetylenic moieties, a variety of amino acids, as well as undecameric peptides and cyclic peptides, have been immobilized onto the dendrimer surface to furnish the final products **77**. Using the catalytic system of $CuSO_4$/sodium ascorbate in DMF mixtures, yields between 43–56% were achieved. Upon applying microwave irradiation, the reaction yields were increased to 96%, which demonstrated the high impact of microwaves on the (positive) reaction progress. Hawker and co-workers[75] have reported similar effects upon reaction of 3,5-dioxybenzyl ether dendrimers **79** with p-(azidomethyl)benzoic acid methyl ester **78**, which yielded the fully substituted dendrimer **80** upon microwave irradiation with $Cu(PPh_3)_3Br$ as catalyst in more than 96% yield as proven by MALDI measurements (Scheme 33). Other dendrimers (such as DAB polyamine dendrimer or Boltorn resin) yielded similar good results under these conditions. Therefore, the method can be used to prepare structurally highly diverse dendritic libraries.

The linking of dendrons up to the 4th generation using click chemistry has been described by Lee et al.[76] (see Scheme 34). Frechet-type dendrons **81** could be linked to the bivalent core (**82**) or another azido-substituted dendron **84** in yields ranging from 84 to 95%, using 5 mol-% of $CuSO_4$/sodium ascorbate in a DMF/water mixture. The trivalent dendrimer **83** or the bivalent dendrimers **85** could be obtained by this reaction, which provides an easy approach to link complexes and, more importantly, large structures. The method can be extended to link two different dendrons together by a click reaction[77] (Scheme 35).

The generation of quasi 'diblock'-dendrons that consisted of a poly(amidoamine) (PAMAM)- and a Frechet-type dendrimer **86** has thus become possible, by the coupling of different dendrons of various size and, of course, with strongly differing properties into one dendritic molecule. The method has been extended to the preparation of symmetrical and asymmetrical PAMAM dendrimers **87** (see Scheme 36), available by coupling the precursor dendrimer **88** with the bivalent azide **89**. A related approach to bivalent dendrimers has been reported by Fokin and co-workers[78] (Scheme 37). Starting from the dendron **90**, where two coumarin dyes are affixed onto a dendron that consists of a Boltorn dendrimer, the carbohydrate building block **91** is attached to the core of the dendrimer, to result in 16 carbohydrate units on the dendrimer surface in **92**.
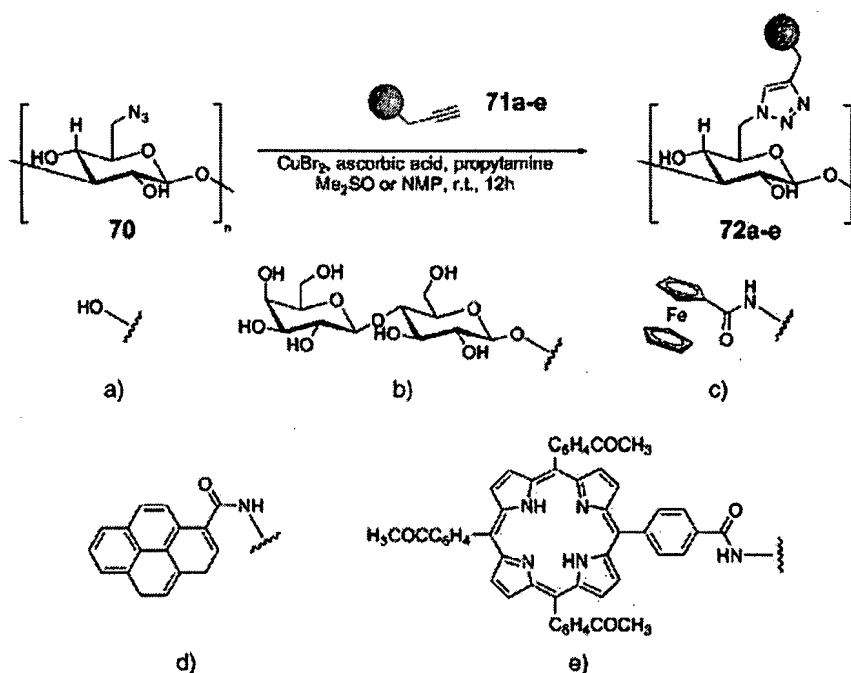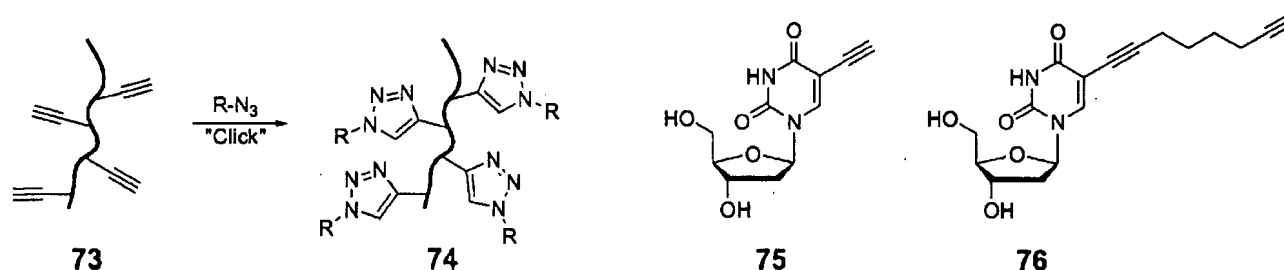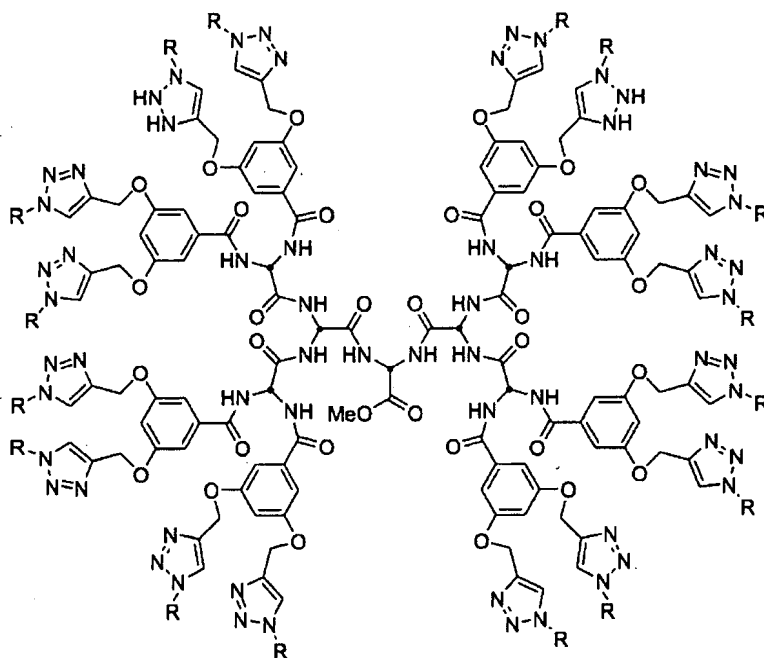

■ Scheme 29.

■ *Scheme 30.*

Frechet and co-workers[79] have used the click reaction to fix dendrons onto polymers displaying terminal acetylenes (Scheme 38). Thus poly(vinyl acetylene) **93** was used as a scaffold for the fixation of dendrons type **90** to yield the dendronized polymer **95**. The reaction works quantitatively for dendrons in the 1st and 2nd generation, and yields coupling products of the 3rd generation in more than 98% yield. Thus this process demonstrates the high steric tolerance of the click reaction even for dendritic structures. From here, certainly it is not far to combine polycondensation processes and click chemistry for the synthesis of dendrimers. Using Frechet's convergent approach for the synthesis of dendrimers (see Scheme 39)[80] the repeated condensation of bivalent acetylene **97** with various azides **96** yields the core-building block **98**. Simple nucleophilic substitution to the azide yields the 'double-edged' building block **99**, which in turn can be condensed with **97** to yield

dendrimer **100**. The formation of higher generations is achieved by repeating the steps, both of which are 100% reactions with respect to yield. Thus dendrimers up to the 4th generation can be constructed, and allow a subsequent end-group modification after the final coupling step.

A similar coupling strategy, which yielded dendrimers with triazoles in the main chain, has been reported by Hawker and co-workers.[81] As shown in Scheme 40, the divergent approach was used to generate the dendron **101**, which could be reacted with an excess of the 'monomer' to generate the dendrimer **102**. Subsequent transformation of the terminal hydroxymethyl-moieties by nucleophilic substitution furnished the octa-azido compound **103**, which can be extended using the same reaction sequence. Dendrimers with purities of 95% could thus be obtained, which is an astonishing number, since divergent methods

■ *Scheme 31.*

77

Scheme 32.

are usually much poorer in their efficiency and fidelity This again demonstrates the high efficiency of the azide/alkyne click reaction.

The methodology of self-coupling of azido and acetylenic building blocks has been used by Voit and co-workers[82] as well as Smet et al.[83] to generate hyperbranched polymers (the so called simple variant of the stepwise-produced dendrimers) (Scheme 41a and 41b). Surprisingly, both authors used the same branched building block **104** as the basic structure for the thermal self-condensation in the sense of a Huisgen-1,3-dipolar cycloaddition process. When **104** is subjected to the thermal process, the hyperbranched polymer **105** ($\overline{M}_n =$ 10 000 $g \cdot mol^{-1}$, which corresponds to $\approx$30 units) is generated. The copper-catalyzed process has been described by Voit and co-workers[82] for another, self-condensing monomer, namely compound **106**, which upon use of the Cu^{II}/sodium ascorbate couple generates a hyperbranched polymer **101**. These processes represent an extremely simple variant to produce dendrimer-like molecules by a simple procedure.

To summarize, the application of the click reaction for the synthesis and derivatization of dendrimers is a highly valuable process, which widens the prospect of structural polymer chemistry. Thus this method will have a large impact in the supramolecular chemistry of polymers as well as their application as central building blocks in material science.

Click Reactions on Surfaces (Including Carbon Nanotubes and C_{60})

One of the very important applications of the azide/alkyne click reaction is its high reactivity in heterogeneous reaction systems, which leads to the prospect of a high efficiency for the derivatization of surfaces and resin materials. In order to run the reaction, the initial key reaction involves an efficient and simple reaction to allow derivatization of a surface functionalized with azido or terminal acetylenes. Table 2 provides an overview of the different surfaces used in conjunction with azide/alkyne click reaction as well as the individual (Cu^I-)catalytic systems. Since the reactions have to be conducted heterogeneously, the exact nature of the solvent system is of importance. In the following context several surfaces are discussed, in which click reactions have been conducted: a) planar surfaces such as self assembled monolayers (SAMs) and glass surfaces, b) polymeric surfaces, c) nanoparticles, and d) micelles and vesicles, as well as e) the functionalization of crosslinked resins bearing terminal azido/alkyne moieties. The subsequent azide/alkyne click reaction then offers an excellent approach to various surfaces exposing functional groups.

Collmann et al.[84] (Scheme 42) were the first to describe the application of SAMs on gold surfaces and the subsequent azide/alkyne click reaction to attach ferrocenes onto the Au surface. They prepared an azido-

78

79

Cu(PPh₃)₃Br
(iPr)₂EtN

80
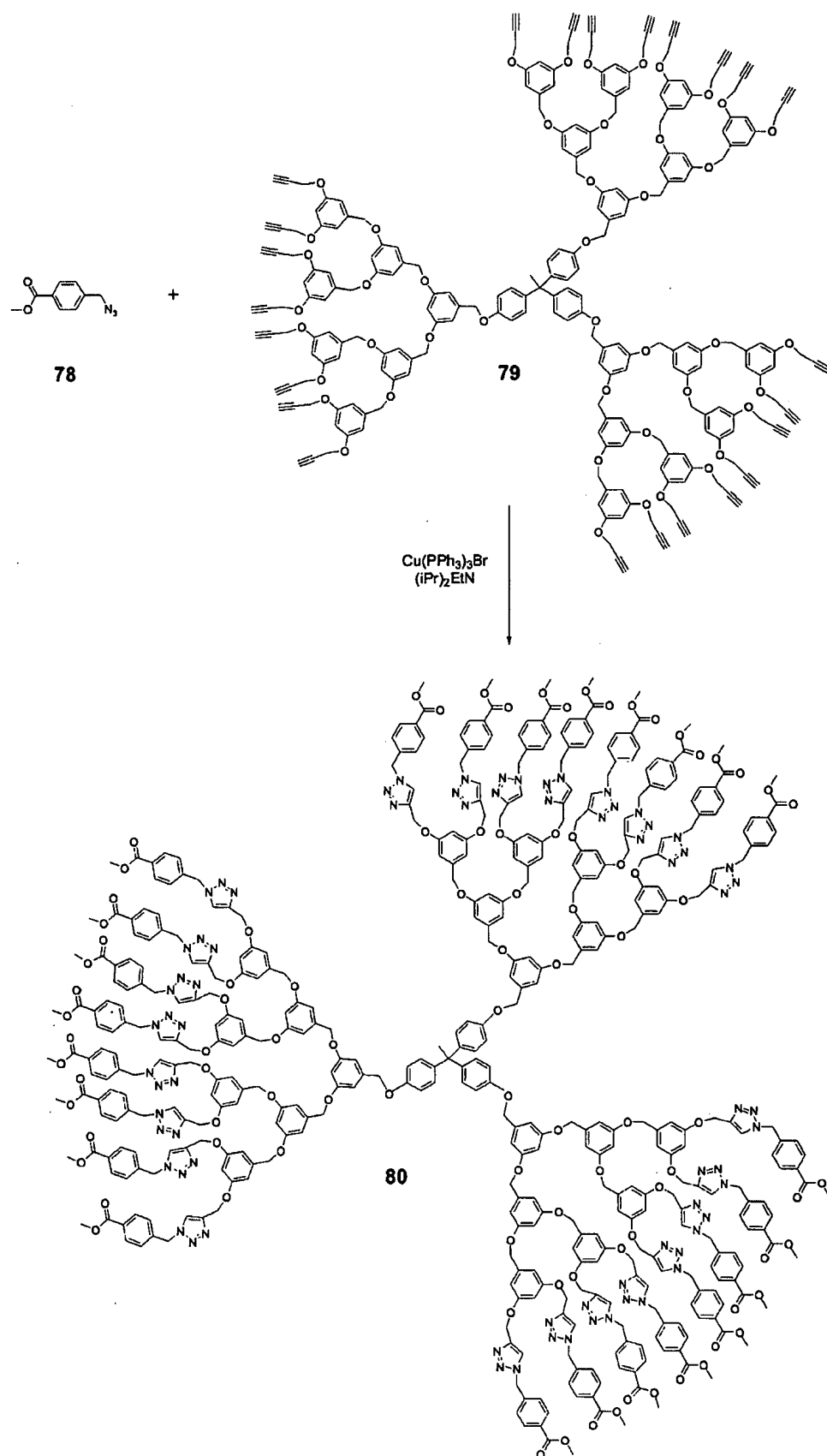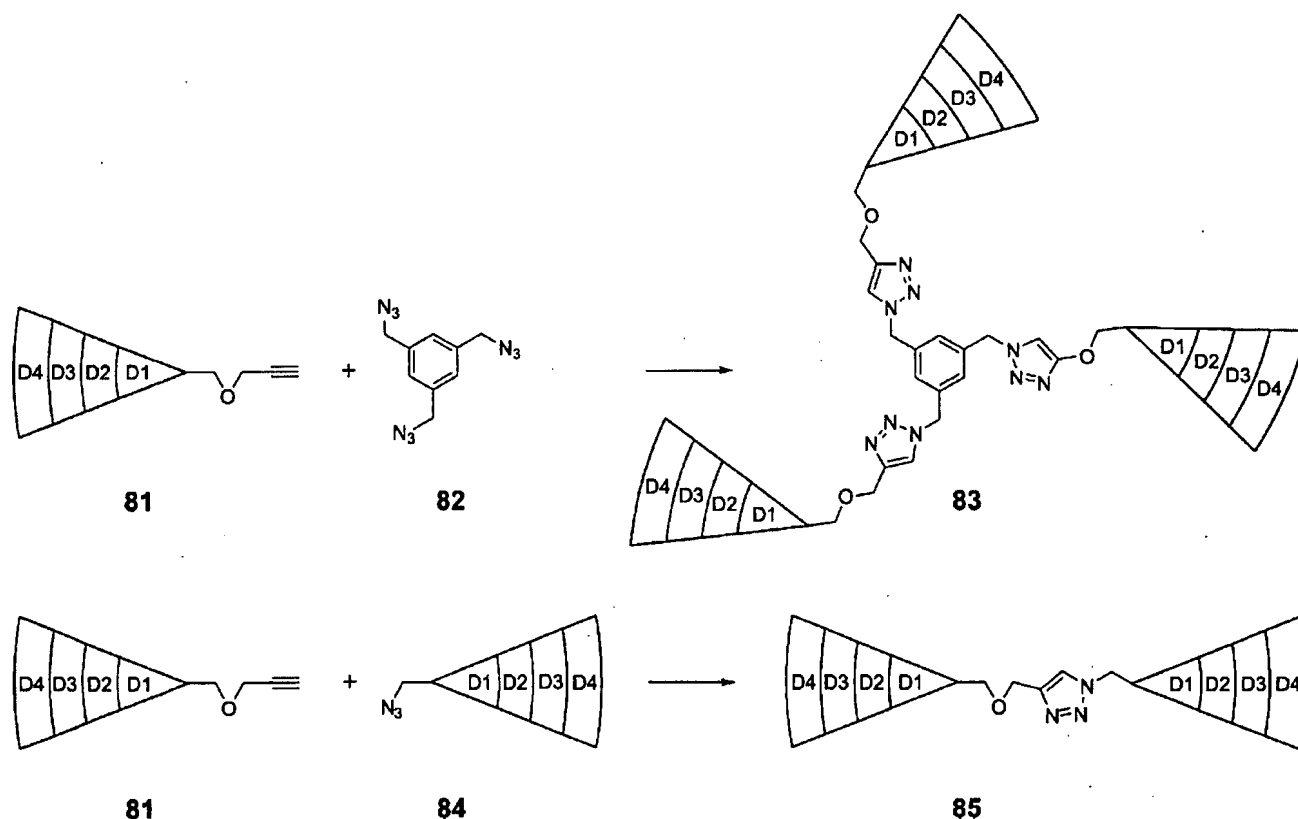
Scheme 33.

■ *Scheme 34.*

functional SAM by adsorption of 11-azidoundecanethiol in a mixture with decanethiol. The subsequent click reaction of the ferrocene-alkynes afforded the corresponding ferrocene-derivatized surfaces. Functionalization was proven by grazing-angle infrared spectroscopy and cyclic voltammetry. The surface coverage was measured as 17% when starting from a monolayer with a coverage of 30%. The density of the final monolayer was dependent on the initial amount of azido moieties present at the surface, thus offering a substrate-size-independent derivatization strategy. In a related publication, the same authors have extended their approach to mixed SAMs on planar Au surfaces.[85] In addition to previously used methods (IR spectroscopy and ellipsometry), X-ray photoelectron spectroscopy (XPS) was used to prove the presence of the attached species. Moreover, a direct, time-resolved measurement of the click reaction onto the electrode surface was achieved. To this purpose, in-situ electrochemical measurements of a ferrocene derivative at a low electrode coverage allowed the determination of the reaction constant of the click reaction as $k = 1 \times 10^3$ $M^{-1} \cdot s^{-1}$, revealing an *extremely high reaction rate* in comparison to other, rapid surface reactions (e.g., the reaction of immobilized cyclopentene and benzoquinone ($k = 220$ $M^{-1} \cdot s^{-1}$), as recently reported by Gawalt and Mrksich[86]). A similar example by the same authors[87] demonstrates

the determination of single electron transfer rates upon variation of the linker between the ferrocene unit and the corresponding Au surface of the SAM (see Scheme 43). Using the click chemistry approach with the Cu^I/TBTA couple, a large variety of ligands with varying spacer length as well as different ligand densities could be affixed to the electrode surfaces, thus enabling a reliable and fast determination of the electron transfer rates.

Choi and co-workers[88] (Scheme 44) have used the inverse approach by presenting an SAM bearing terminal acetylenic moieties. They too applied the click reaction in aqueous reaction systems using $Cu^{II}SO_4$ and sodium ascorbate as the catalytic system. The attachment of the azides **108**, **109**, and **110** was proven by polarized infrared external reflection spectroscopy (PIERS) and XPS. Most importantly the attachment of the azido-modified nucleoside **109** onto the surface was demonstrated, thus proving the ability to attach molecules of biological significance onto SAM surfaces.

An example of a purely thermal Huisgen 1,3-dipolar cycloaddition has been reported by Lummerstorfer and Hoffmann[89] using chemistry on SAMs derived from silicon and silica surfaces (Scheme 45). Since no catalysts were used, the reaction needs a temperature of 70 °C in order to obtain a significant attachment of functional moieties onto the surface. An advantage of this uncata-
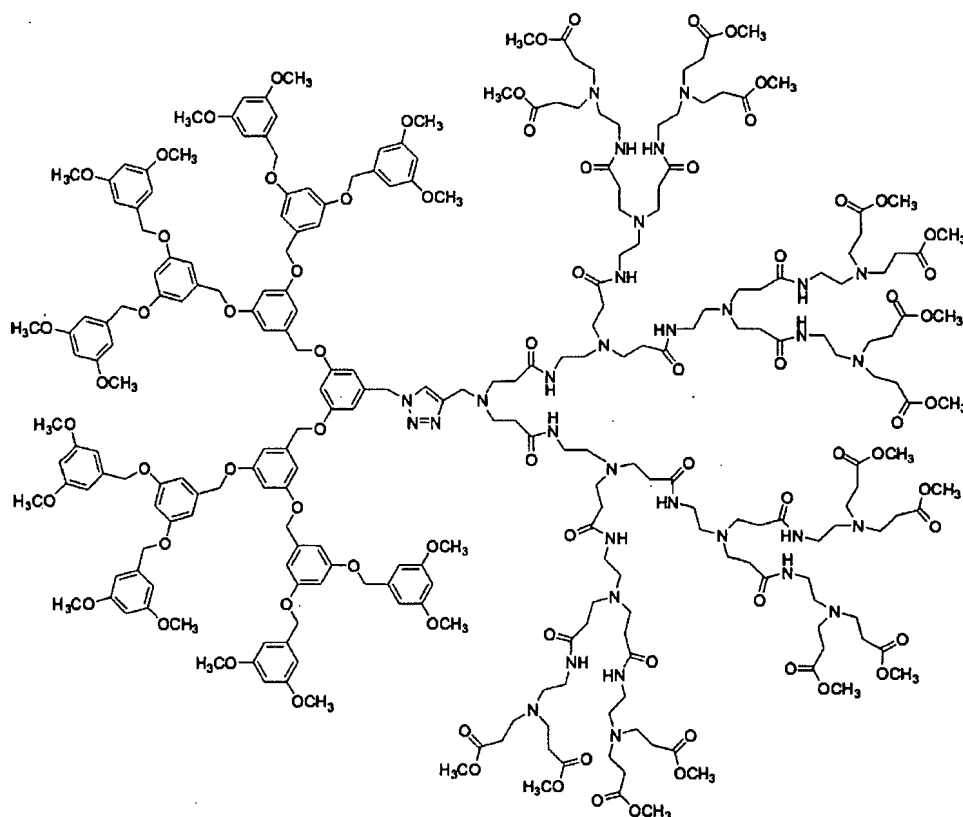
**86**

■ *Scheme 35.*

lyzed process is the attachment of the bisubstituted, activated acetylene dicarbonic acid and its derivatives onto the SAM.

A recent example of the click reaction onto SAM surfaces was reported by Binder and co-workers[90] (Scheme 46) which demonstrated the attachment of highly polar hydrogen-bonding systems **111** onto the ω-azido ligand **112** bound to SAMs. Again, the reaction scales with the amount of azido moieties bound to the surface, thus demonstrating the broad applicability of this reaction for the attachment of supramolecular receptors. A coverage of the SAM with the functional moiety **111** could thus be achieved, and ranged from only a few mol-% up to a full coverage, as determined by atomic force microscopy (AFM) detection of the supramolecular ligand sticking out of the planar surface. The system described works in aqueous as well as non-aqueous environments using $Cu^I Br(PPh_3)_3$ as catalyst. A critical point concerned the use of a minimal amount of triphenyl phosphine, which can also act in a competing reaction that leads to a destruction of the azido moieties in a Staudinger-type reaction. The supramolecular ligand in turn could be used to assemble nanopar-

ticles and other nanometer-sized objects onto the surface by a specific supramolecular interaction. The method has been extended for the immobilization of various complex carbohydrates onto Au surfaces by SAMs (see Scheme 47).[91] After first immobilizing a ω-acetylenic substrate onto the SAM, various azido sugars **113** as well as the azido glycol **114** were reacted onto the surface using $CuSO_4$/sodium ascorbate/ethanol/water mixtures. Chaikof and co-workers[91b] have used silicon substrates and an additional, reversible Diels-Alder linker, able to be cleaved reversibly by thermal activation. In both cases, the ligand attachment was proven by antibody binding onto the carbohydrate moieties by surface plasmon resonance (SPR), which provides an important platform for the development of sensor-based detection and screening systems.

Two recent examples have combined the microcontact-printing of Au SAMs with the click reaction. One example has been mentioned already in the above polymer section, and relies on terminal acetylenes in poly[(4-ethynyl-*p*-xylylene)-*co*-(*p*-xylylene)] as shown in Scheme 28.[58] The polymer was prepared as a thin film by CVD of
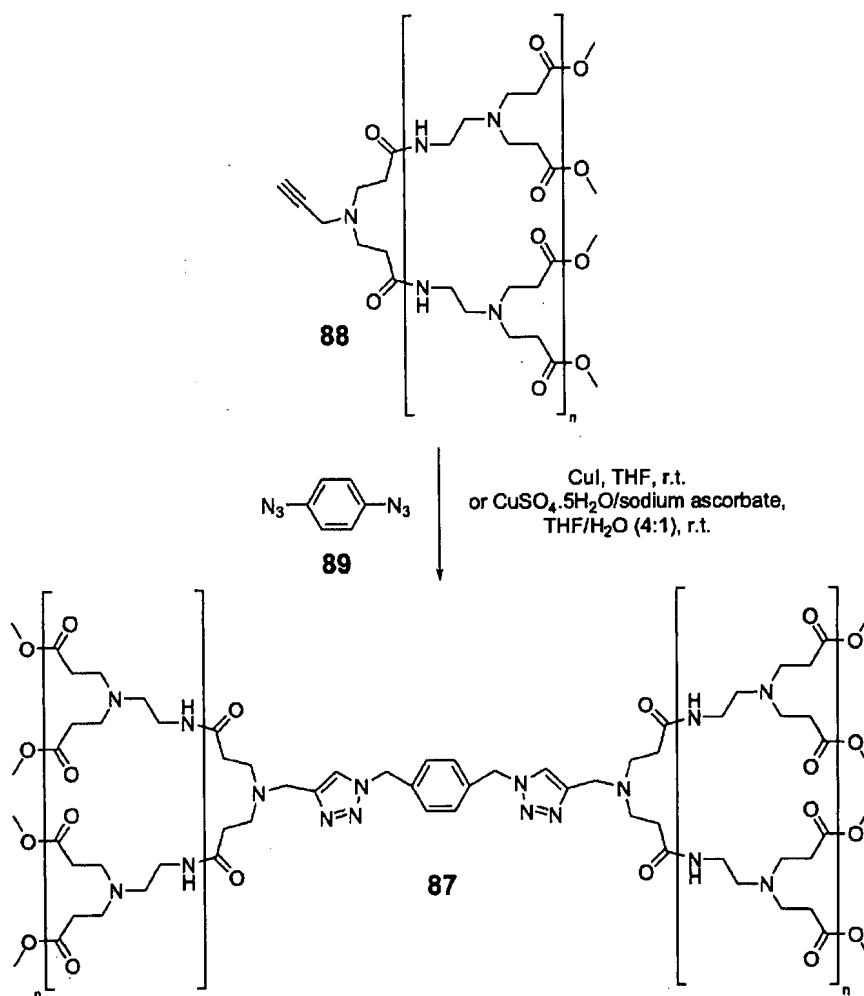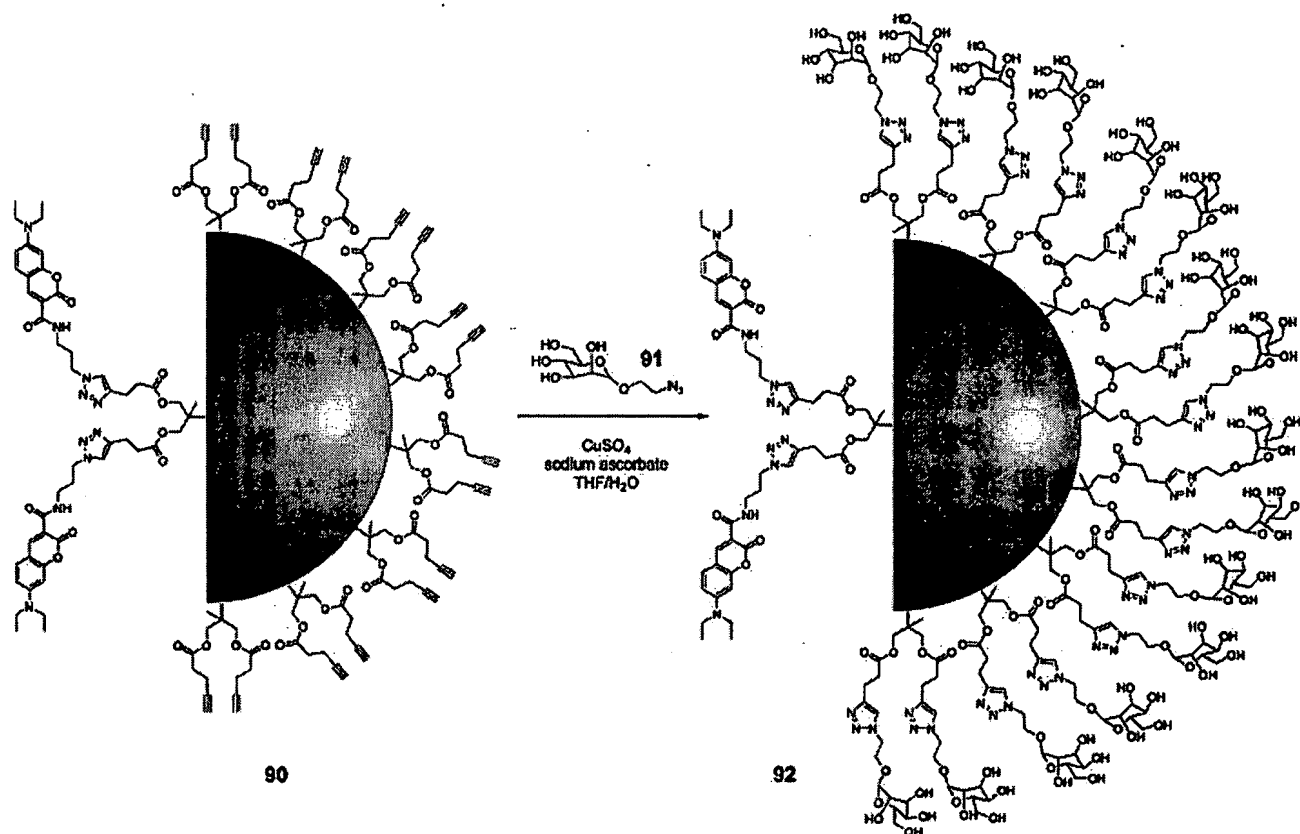
■ *Scheme 36.*

ethynyl[2,2]paracyclophane, which resulted in an ≈50 nm thick film. Conventional microcontact printing supplied $CuSO_4$ to specific regions of the polymer film, to result in a local click reaction between an ω-azido-biotin and the sidechain acetylenic moieties of the polymer. A similar example on silicon dioxide surfaces has been demonstrated by Reinhoudt and co-workers.[92a] As shown in Scheme 48, the initial SAM of the ω-bromo-siloxane monolayer **115** was reacted with sodium azide in DMF at 70 °C, furnishing the azido-modified SAM, similar to the work reported by Lummerstorfer and Hoffmann.[89] A silicon stamp (bearing line features, immersed with 5 × 10^{-3} M octadec-1-yne) was then applied directly to the wafer **116** for 15 min at room temperature with a load of 35 g *without the use of a copper catalyst.* As judged by AFM (by detecting the height differences as a result of the applied alkyne) as well as by XPS (by relating the intensities of the C 1s peak versus the N 1s peak), the attachment of the alkyne to yield surface **117** was proven. This,

therefore, represents the first example of a low temperature, very fast attachment reaction without coupling reactions. Moreover, it demonstrates that the azide/alkyne click reaction is working at moderate reaction temperatures simply as a result of the close proximity of the azide and alkyne reactive functionality. An important example of a click reaction mediated by an AFM tip has been reported by Bunz and co-workers.[92b] Most importantly, the reaction between a bivalent dialkyne derivative and a bivalent diazide has been accomplished by heating a specific region of a film by action of an AFM tip to ≈250 °C, thus inducing the formation of conjugated fluorescent polymers within a lithographic process.

The direct, non-oxidative modification of pure Si(1,1,1)-surfaces by click reactions has been achieved by Heath and co-workers.[93a] Thus a Si surface that presented terminal acetylenes was prepared by a short reaction sequence featuring PCl_5 activation (to yield a chloro-functional surface) and a subsequent nucleophilic displacement
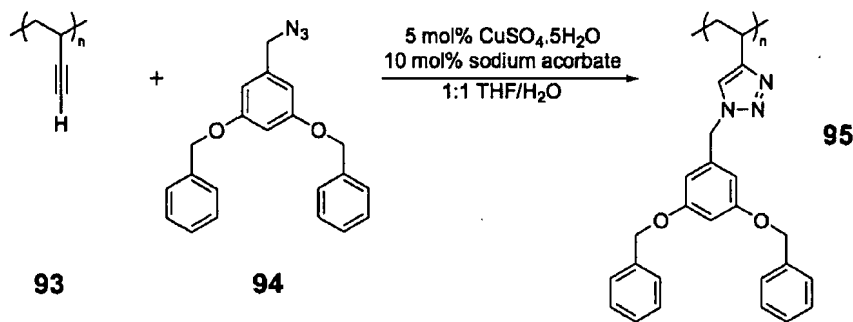
■ Scheme 37.

reaction by sodium acetylide, to result in the surface **118**
(see Scheme 49). The click reaction (Cu^{II}SO_4/sodium
ascorbate/DMF/12 h) in turn enabled the attachment of
electroactive benzoquinones **119** to yield surfaces **120**. The
benzoquinones could be cleaved off electrochemically, to
enable the subsequent derivatization of the so-obtained
amino-functionalized surfaces with other ligands (e.g.,
ferrocenes). Another report[94] on the modification of
porous silicon started from a Si-H surface and a subsequent
attachment of 1-vinylhept-6-yne, which in turn enabled
the click reaction of various biologically important

molecules. Both methods open an interesting pathway
to functionalize silicon wafers in their native, pure silicon
state. Using layer-by-layer (LbL) deposition, Caruso and
co-workers[93b] have reported a similar example to
sequentially deposit sidechain-modified acetylenic and
azido-modified polymers onto a surface, by 'clicking' them
under formation of the triazole bridge.

Certainly, there is only a small difference between
planar surfaces and curved surfaces, putting nanoparticles,
as well as vesicles and other, related colloidal structures in
the limelight of the click-based derivatization process. Two
recent examples have demonstrated
the versatility of the click reaction on
nanoparticles (NPs), namely on Au
NPs[95] as well as on CdSe NPs and
polyhedral oligomeric silsesquioxanes
(POSS).[96] The transformation of the
conventional Au monolayer chemistry
to those of NPs is demonstrated in
Scheme 50. Thus a conventional ω-
azido-alkyl ligand is generated on Au
NPs (diameter ≈2.0 nm) by a nucleo-
philic displacement reaction (bromo-
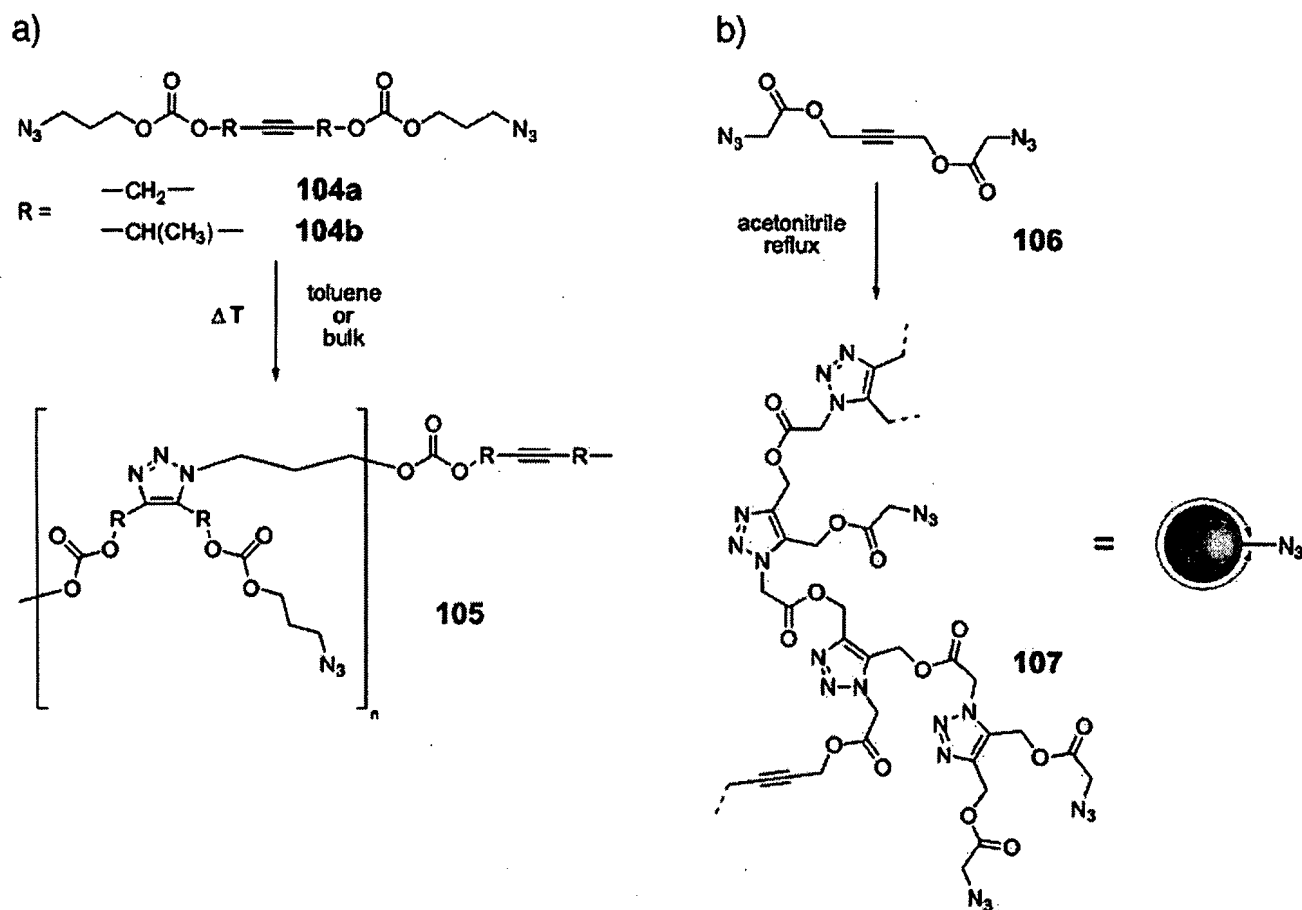methyl/azidomethyl exchange) and

■ Scheme 38.
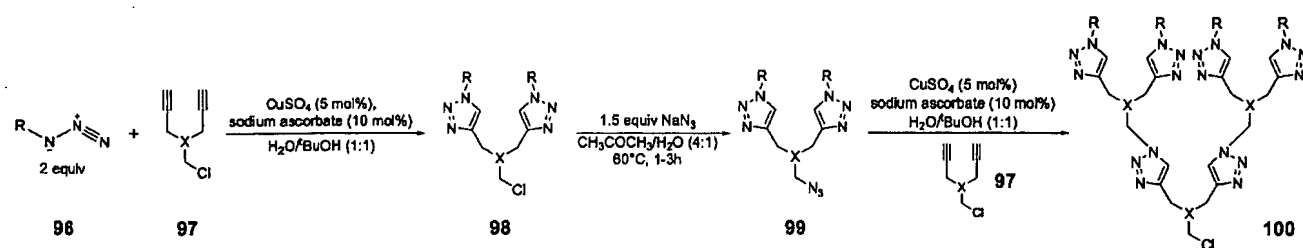
Scheme 39.

Scheme 40.

a)
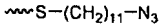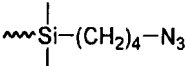
b)
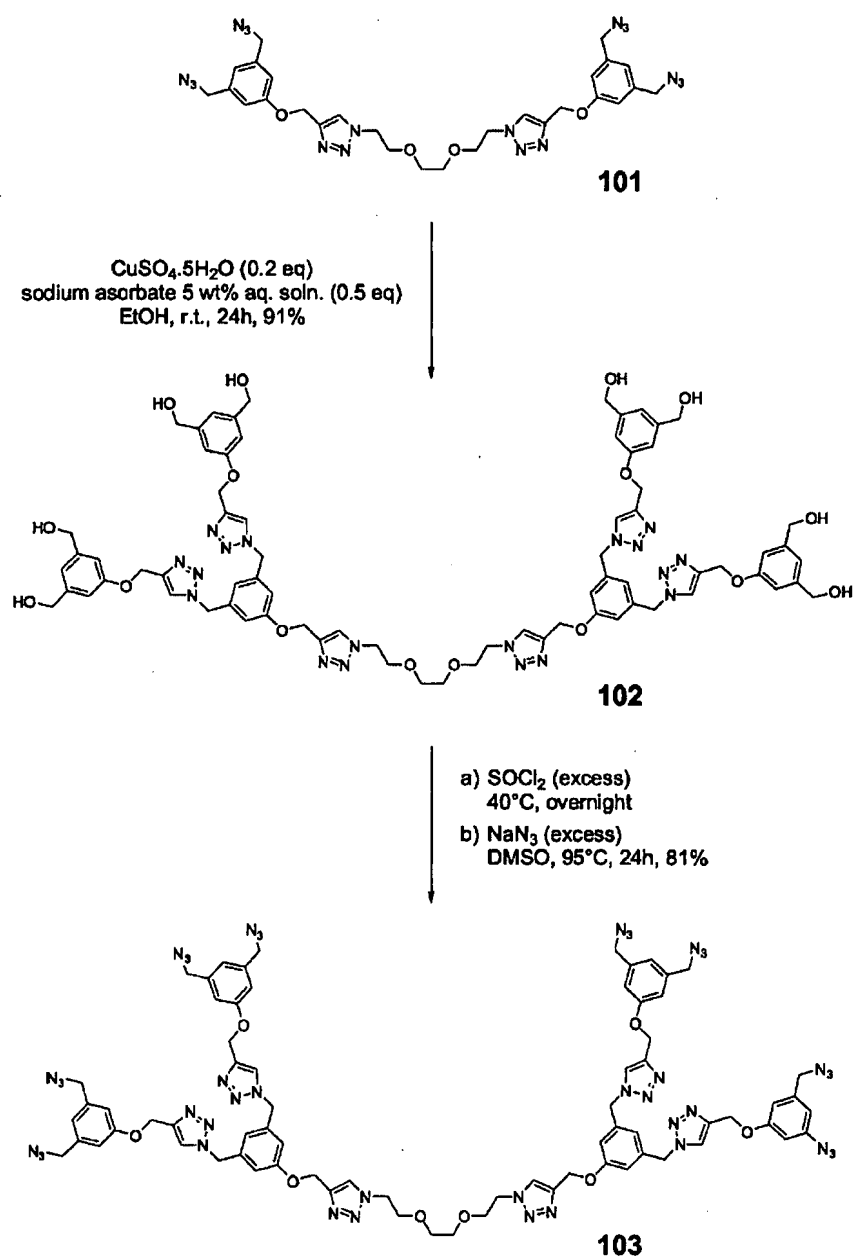
■ *Scheme 41.*
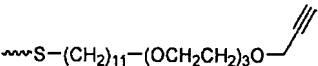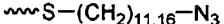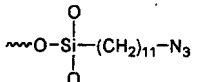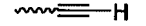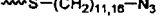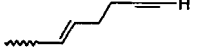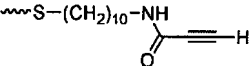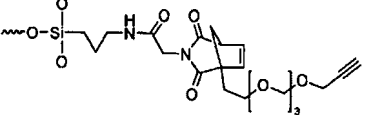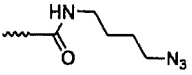
subsequent click reaction with acyl-activated acetylene ligands **121**. The surface attachment was proven by IR spectroscopy as well as by cyclic voltammetry.

Click reactions on micelles and vesicles (liposomes) constitute another issue, addressed recently by several authors. The derivatization of both systems in an easy, room temperature-controlled process with (biorelated) ligands is important, mainly because of their dynamic properties, which prevents reac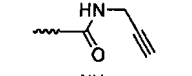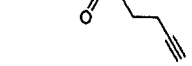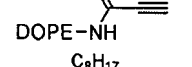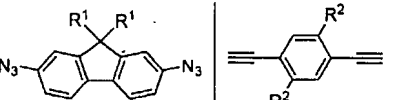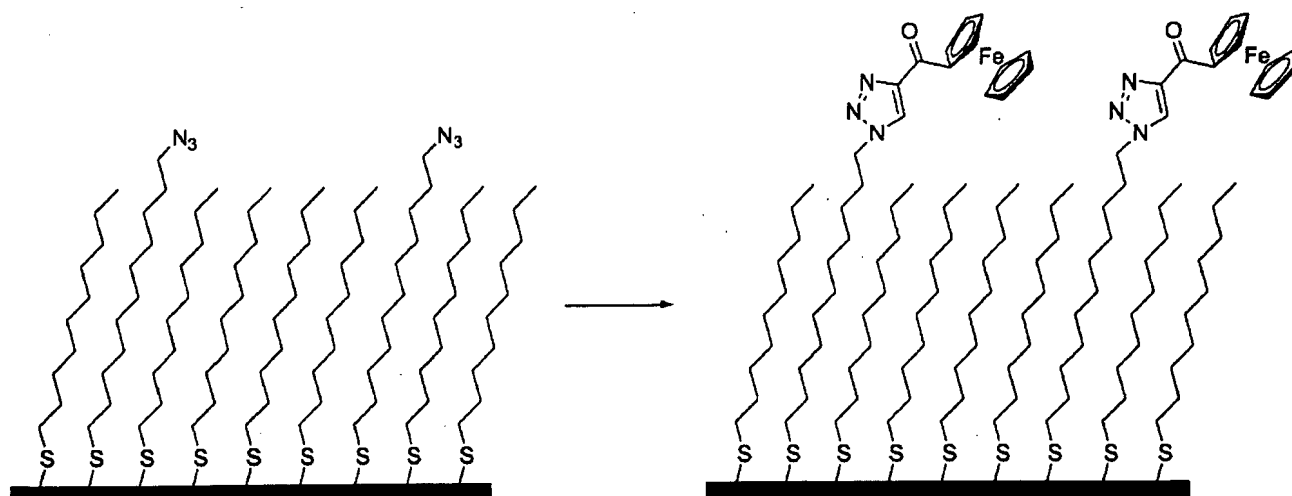tions at higher temperatures. Hawker and co-workers[97] have reported on the modification of block-copolymeric micelles (BCP micelles) by use of the click reaction. A diblock copolymer consisting of a hydrophilic shell and a hydrophobic, PS interior was prepared by NMP. After micelle formation, the outer, carboxylic acid moieties were reacted with either propargylamine or 3-azido-propylamine to yield the corresponding alkyne- or azide-modified BCP micelles. The derivatization process was subsequently performed on these micelles to yield the correspondingly modified BCP micellar surfaces.

Vesicular bilayers represent another surface whose derivatization is important. Two different examples have

been described, both retaining the (usually fragile) structure of the vesicles. Schuber and co-workers[98] have used small unilamellar vesicles (SUVs), composed of dipalmitoyl phosphatidyl choline (DPPC), dipalmitoyl phosphatidyl glycerol (DPPG), and cholesterol (Scheme 51) with an outer shell of terminal acetylenes, achieved by incorporation of the unnatural lipid **125** in amounts of 10 mol-%. Using the classical click conditions, carbohydrate-bearing substrates (e.g., **122**) were coupled to the SUV's surface with the $Cu^{II}SO_4$/sodium ascorbate couple at a pH of 6.5. Despite an $\approx$50-fold increase in diameter of the derivatized vesicles **124** in size during the coupling reaction, no leakage of the liposomes was observed, proving the mildness of the reaction without disturbance of the bilayer membrane. The other example has been reported by Kros and co-workers[99a] using a dioleoylphosphatidyl ethanolamine (DOPE)-modified lipid **126** with an acetylenic head group (see Scheme 52). Fluorescence resonance energy transfer (FRET) methods were used to prove the attachment of ligand **128** by Cu^IBr-catalysis onto the surface of the liposome, which enabled the transfer of energy between the attached dye and another dye incorporated

■ *Table 2.* Overview of click reactions on surfaces.

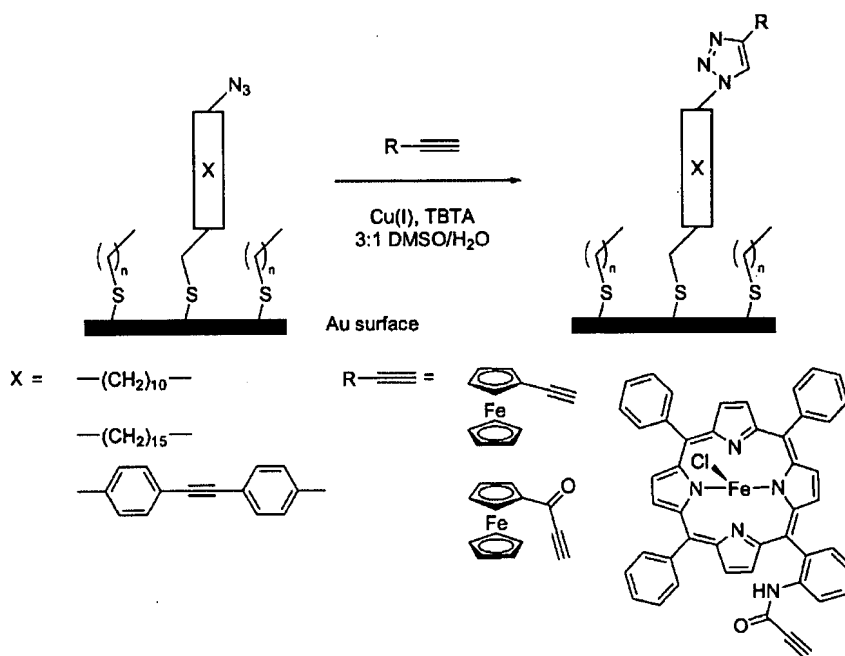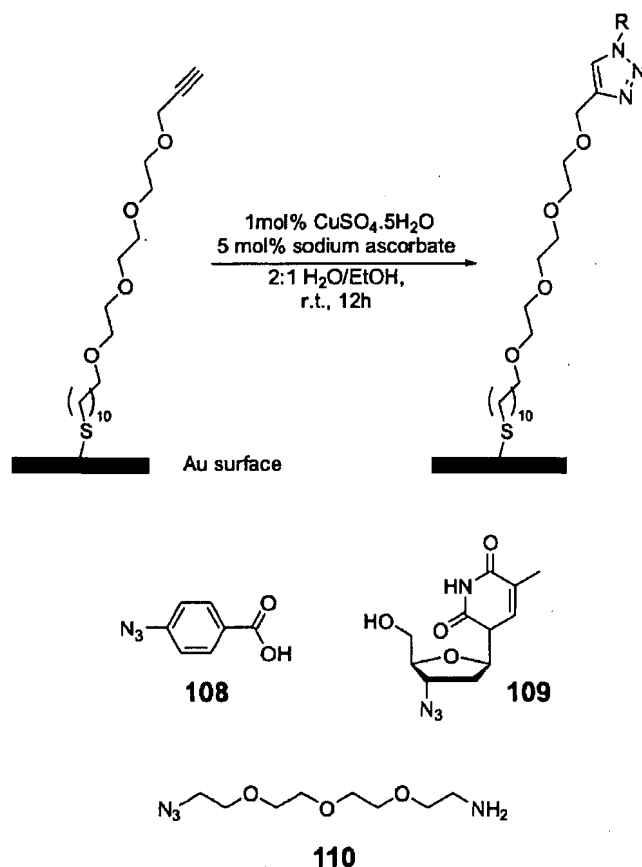| Entry	Surface/type	Chemical structure on surface	Investigated catalytic systems	Ref.
1	SAM on Au/planar	∿∿S—(CH$_2$)$_{11}$—N$_3$	CuSO$_4$·5H$_2$O/sodium ascorbate/H$_2$O/EtOH	[84]
2	SAM on SiO$_2$/planar	∿∿Si—(CH$_2$)$_4$—N$_3$	thermal/70 °C/neat	[89]
3	SAM on Au/planar	∿∿S—(CH$_2$)$_{11}$—(OCH$_2$CH$_2$)$_3$O—	CuSO$_4$·5H$_2$O/sodium ascorbate/H$_2$O/EtOH	[88]
4	SAM on Au/planar	∿∿S—(CH$_2$)$_4$—N$_3$	CuSO$_4$·5H$_2$O/sodium ascorbate and (Ph$_3$)$_3$Cu(I)Br/H$_2$O/EtOH	[90]
5	SAM on Au/planar	∿∿S—(CH$_2$)$_{11,16}$—N$_3$	CuSO$_4$·5H$_2$O/sodium ascorbate/H$_2$O/EtOH and DMSO/H$_2$O	[85]
6	SAM on SiO$_2$/planar	∿∿O-Si—(CH$_2$)$_{11}$—N$_3$	no catalyst/r.t./μ-contact printing	[92a]
7	Si(111) surface/planar	∿∿≡—H	CuSO$_4$/sodium ascorbate/DMF/r.t.	[93a]
8	layer by layer (LbL)film of polymer	∿∿≡—H	CuSO$_4$/sodium ascorbate/pH = 3.5	[93b]
9	SAM on Au/planar	∿∿S—(CH$_2$)$_{11,16}$—N$_3$	TBTA CuBF$_4$/hydroquinone/DMSO/H$_2$O	[87]
10	porous Si	∿∿≡—H	CuSO$_4$/ascorbic acid, MeCN/tris-buffer/pH = 8.0, r.t.	[94]
11	SAM on Au/planar	∿∿S—(CH$_2$)$_{10}$—NH—C(O)≡—H	CuSO$_4$/sodium ascorbate/H$_2$O/EtOH	[91a]
12	SAM on glass	∿∿O-Si—NH—(maleimide)—O[O]$_3$O—≡	CuSO$_4$·5H$_2$O, TBTA,TCEP/PBS-buffer/t-BuOH, 4 °C	[91b]
13	SAM on Au NPs 1.8 ± 0.4 nm	∿∿S—(CH$_2$)$_{11}$—N$_3$	dioxane/hexane/r.t.	[95]
14	crosslinked BCP micelle by NMP	∿∿HN—C(O)—N$_3$	CuSO$_4$·5H$_2$O/sodium ascorbate/H$_2$O	[97]
15	SUV 90–130 nm	∿∿HN—C(O)—≡ / ∿∿NH—C(O)—≡	CuSO$_4$/sodium ascorbate/HEPES-buffer/pH = 6.5	[98]
16	SUV 110–120 nm	DOPE—NH—C(O)—≡	CuBr/H$_2$O	[99]
17	CdSe NP	O=P(C$_8$H$_{17}$)(C$_8$H$_{17}$)—N$_3$	CuBr	[96]
18	polymer layer	N$_3$—(fluorene R^{1}R^1)—N$_3$ / ≡—(benzene R^{2}R^2)—≡	AFM-tip/225 °C	[92b]

■ *Scheme 42.*

into the membrane itself. Both examples demonstrate that liposome surfaces in future may be broadly derivatized using this method, moreover allowing the exclusive modification of the outer side of the liposomal surface.

Another important development concerns the attachment of ligands for the subsequent solid phase synthesis of peptides and their corresponding ligation products with carbohydrate ligands. Thus Meldal and co-workers[5] have attached a variety of ligands onto resins modified with acetylenic ligands. A hydroxymethylbenzoic acid (HMBA)-modified poly(ethylene glycol acrylamide) (PEGA$_{800}$) resin

was modified with a peptidic anchor bearing a terminal propargylglycine moiety. Subsequently a large variety of azides (e.g., 2-deoxy-2-azido carbohydrates, α-azido acids,[100] adamantane, and carboxylic acids)[100] were coupled to the resin in yields >95%. The chemistry was shown to be compatible with conventional amino acid protecting groups. In addition, a high 1,4-regioselectivity was observed on using the Cu^{I} catalyst. A related concept has been developed by Gmeiner and co-workers[101] starting from a conventional Merrifield resin [(chloromethyl)styrene] treated with sodium azide, thus furnish-
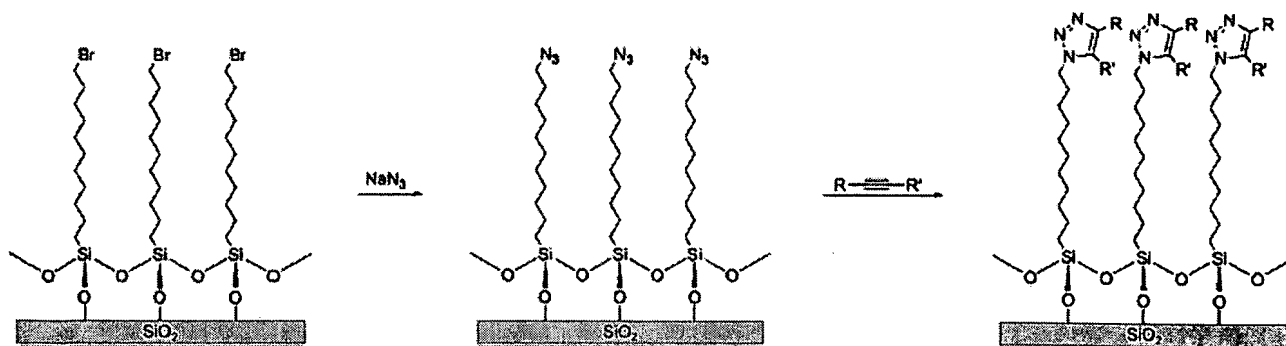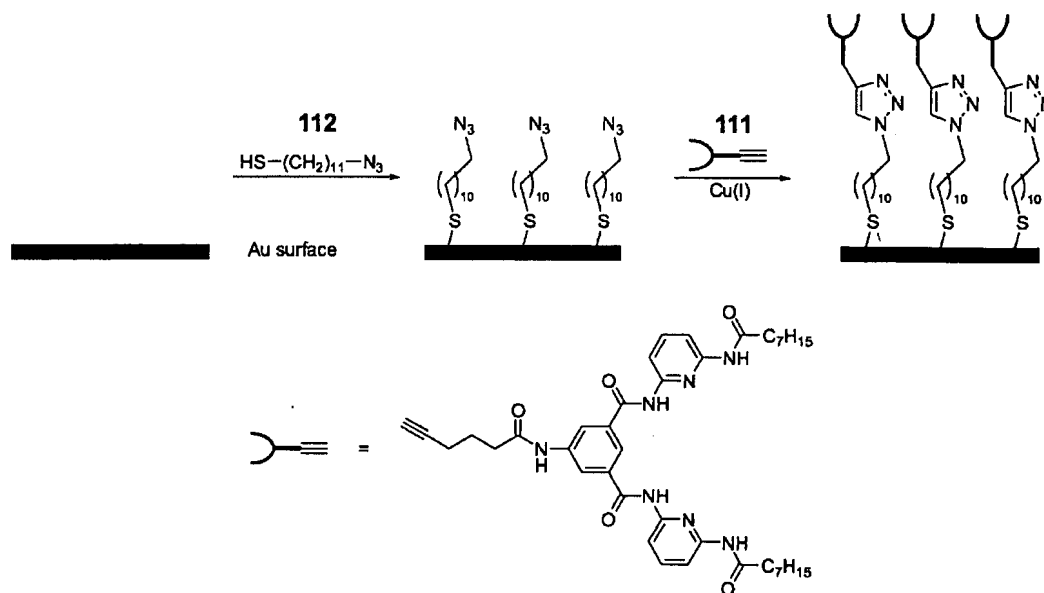

■ *Scheme 43.*

■ *Scheme 44.*

chemistry approach. A related approach for the generation of linkers sensitive to conditions employed under MALDI measurements was developed by Finn and co-workers,[102] which relied on a combination of cycloreversion processes and click reactions.

An interesting application of 1,3-dipolar cycloaddition reactions has been demonstrated using carbon nanotubes and C_{60}.[103] As discovered recently, the use of highly reactive dipolarophiles (such as azomethine ylides,[104] nitrile imines,[105] and nitrile oxides[106]) leads to cycloaddition reactions onto the surface of single-walled carbon nanotubes (SWCNTs).[107] However, slow reactions are often observed, which lead to reaction times in the range of several days. Therefore, the use of the azide/alkyne click reaction has been applied to fix a telechelic PS polymer by a grafting-to approach onto the surface of SWCNTs (see Scheme 53).[108] Since a direct, thermally induced reaction between the azide and the extended π-system of the SWCNT by nitrene-mediated addition is to be avoided, the Cu^I-mediated process was investigated, using an azido-telechelic PS polymer (prepared by ATRP according to previously mentioned methods). SWCNTs functionalized with terminal acetylenic moieties (prepared by a solvent free method that employed a diazotation reaction) were reacted with the azido-telechelic PS **129** ($\overline{M}_n = 2\,010$ to $8\,620$ g·mol^{-1} with $\overline{M}_w/\overline{M}_n = 1.09$ to 1.13) by use of the Cu^IBr/bipyridine system. As demonstrated extensively by solubility changes and thermogravimetric analysis, a significant attachment of the PS polymer was achieved. The structural integrity of the SWCNTs was also proven by Raman spectroscopy to reveal the absence of thermally induced nitrene reactions. In comparison to SWCNTs prepared by the nitrene-insertion methods, enhanced solubility as a result of the absence of the highly reactive nitrene moieties (which leads to additional, non-selective crosslinking) was observed.

ing the azido-functionalized resin. This was linked to several formyl-aryloxypropynes to generate the corresponding 1,2,3-triazoles in more than 95% yield using Cu^II/DIPEA in THF at 35 °C. This allows a traceless-linker approach to generate fixed amide libraries, which can be cleaved from the resin using trifluoroacetic acid (TFA). This opens a new approach to traceless linkers onto Merrifield resins and demonstrates the high efficiency of the click
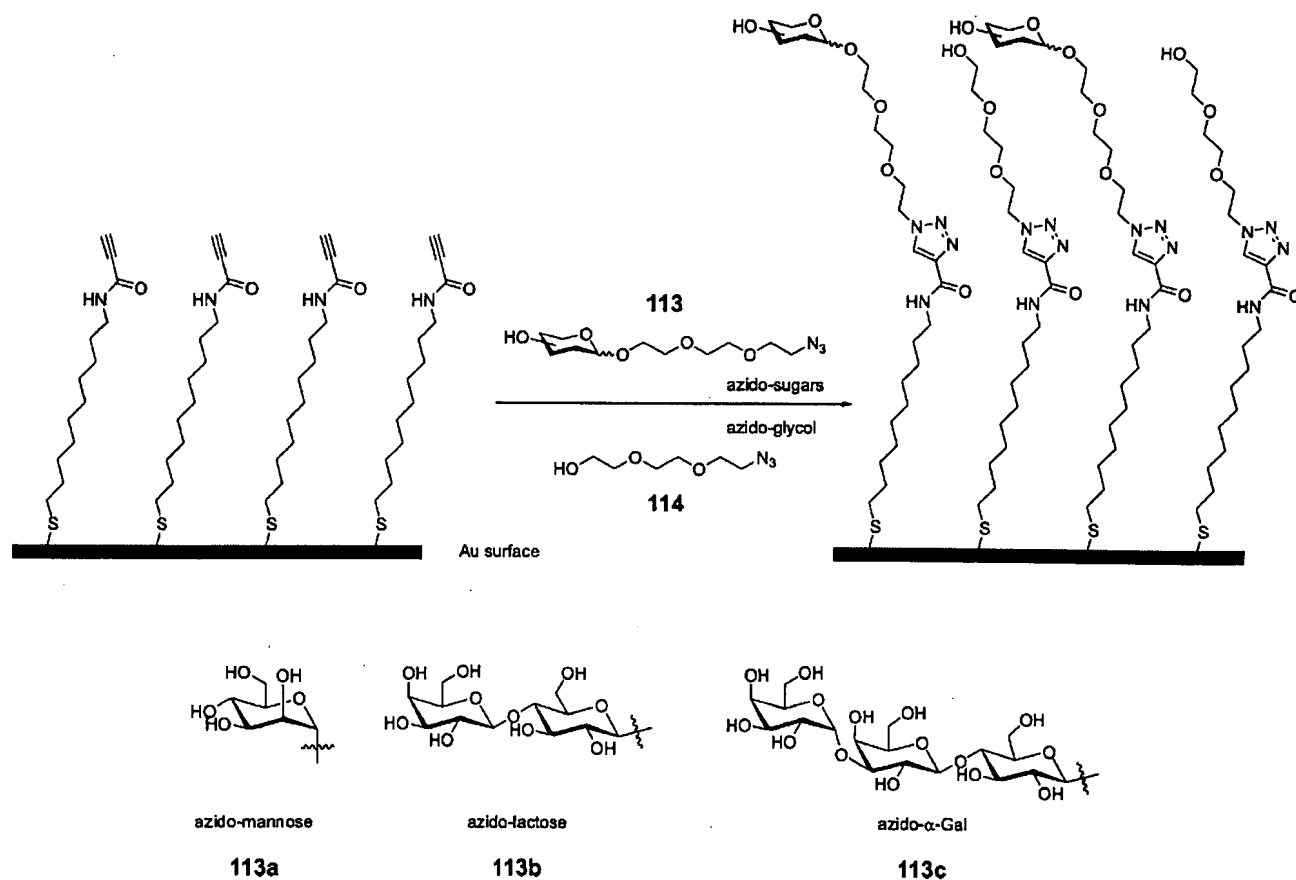
To summarize, the click reaction works excellently on planar (two-dimensional) as well as on crosslinked three-dimensional (resin-type) surfaces, to furnish an efficient
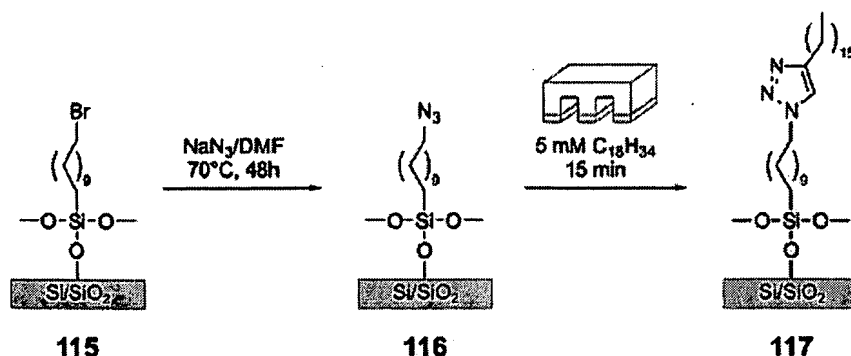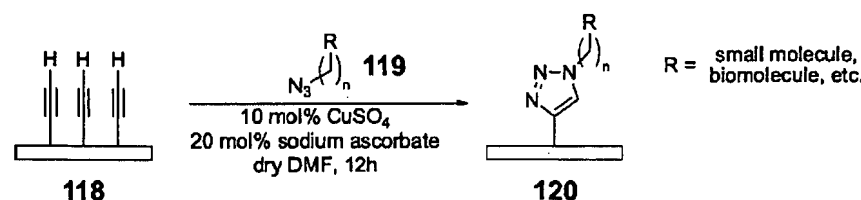

■ *Scheme 45.*

■ *Scheme 46.*

azido-mannose azido-lactose azido-α-Gal

113a **113b** **113c**

■ *Scheme 47.*

Scheme 48.

Scheme 49.

$R = $ small molecule, biomolecule, etc.

by Meldal and co-workers[5] who used the click reaction on peptides during solid-phase synthesis. Based upon this knowledge, Ghadiri and co-workers[110] used the click reaction to generate cyclic peptides.

The purely thermal 2,3-thermal Huisgen reaction was used by Ju and co-workers[65] to link rhodamine dyes to the end of oligonucleotides. The starting oligonucleotides displayed a terminal amino group, which in turn could be subjected to the thermal dipolar cycloaddition process at 80 °C with an acetylene-modified rhodamine dye. An impressive example of the completeness and versatility of the azide/alkyne click reaction has been presented by Finn and co-workers[111] upon modifying all sixty positions of the tobacco mosaic virus. After the generation of a virus-derivative displaying sixty azido, or acetylenic bonds, the final coupling with a rhodamine B dye yields the attachment of sixty moieties onto the surface of the labeled virus. The catalyst system used was based on $Cu^{II}SO_4$/tris(carboxyethyl) phosphine (TCEP)/catalyst/Cu^0. Both systems yielded an attachment efficiency of 100% in a solvent system based on phosphate buffer (plus 5% *t*-butyl alcohol) at a pH of 8.0.

The biolabeling in living systems was demonstrated by Tirell and co-workers[112] by incorporating the unnatural amino-acids azido-alanine, azidohomoalanine, azidonor-valine, and azidonorleucine into living cells. Reporting and quantification of the incorporation was subsequently done by 'clicking' an acetylene-modified biotin ligand onto the surface of the E-coli bacteria, which displayed the azido moieties. Ultrapure Cu^IBr (purity 99.999%) was found to be superior in catalyzing the click reaction. A related approach to the use of activity based protein profiling (ABPP) has been reported by Cravatt and co-workers,[113] which relied on ABPP as a chemical proteomic method, which employs active site-directed probes to simultaneously visualize the changes in activity of proteins in a cellular environment.[114] To summarize, the combination of azide/alkyne click chemistry demonstrates a new approach to the profiling of enzymatic active sites as well as the search for new binding structures onto active protein sites. Clearly, an absolutely randomized approach (without knowledge of the enzyme's active site) as well as an at least partially directed

and general approach for their functionalization. A large number of surfaces, however, have not been investigated up to now, thus leaving an ample field for further investigations on the versatility of this reaction.

Click Reactions onto Biomolecules, Cells, and Viruses

Certainly the click reaction is particularly apt for the ligation of artificial ligands onto biomolecules, since this reaction is especially useful to work under conditions in which the structural integrity of peptides, proteins, carbohydrates, and assemblies derived there from is preserved. Thus besides conventional linking methods (e.g., disulfide exchange, amide linkage, reductive aminations, Staudinger-type ligations),[109] this method definitely is an important contribution for biological labeling. Recent reviews deal with this topic,[38] therefore, the focus here is on new literature. One of the first examples to demonstrate the practicability of the azide/alkyne click reaction was provided
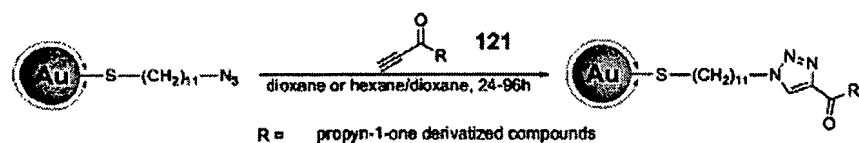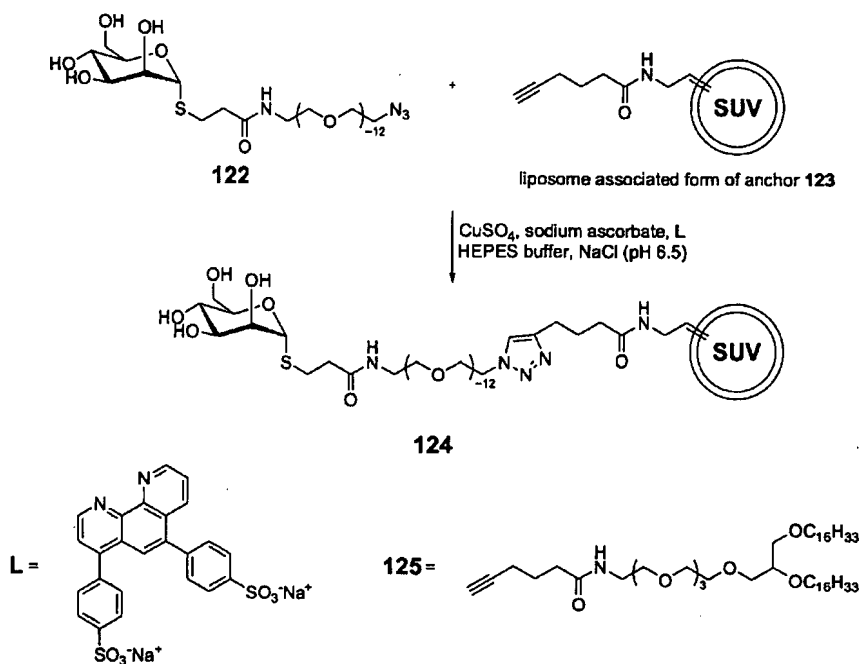

R = propyn-1-one derivatized compounds

Scheme 50.

122

liposome associated form of anchor **123**

CuSO$_4$, sodium ascorbate, L
HEPES buffer, NaCl (pH 6.5)

124

L =

125 =

■ *Scheme 51.*

128

FRET

DOPE—N $=$

126

DOPE—LR $=$

127

■ *Scheme 52.*

DOI: 10.1002/marc.200600625

■ *Scheme 53.*

approach is viable, to screen dynamic combinatorial libraries. Therefore, this method points to the future for studying associative phenomena, possibly also in supra-molecular chemistry and materials science.

Conclusion and Outlook

During the past several years, click reactions have already demonstrated their efficiency and use in material chemistry and science, in particular in the field of polymer science. A plethora of chemists, tired of the extensive use of protecting-group strategies and insufficient reaction progress, have been searching for click-type reactions for decades. Now, with the advent of the azide/alkyne-1,3-dipolar cycloaddition click reaction, at least a visible step in this direction has been achieved, whic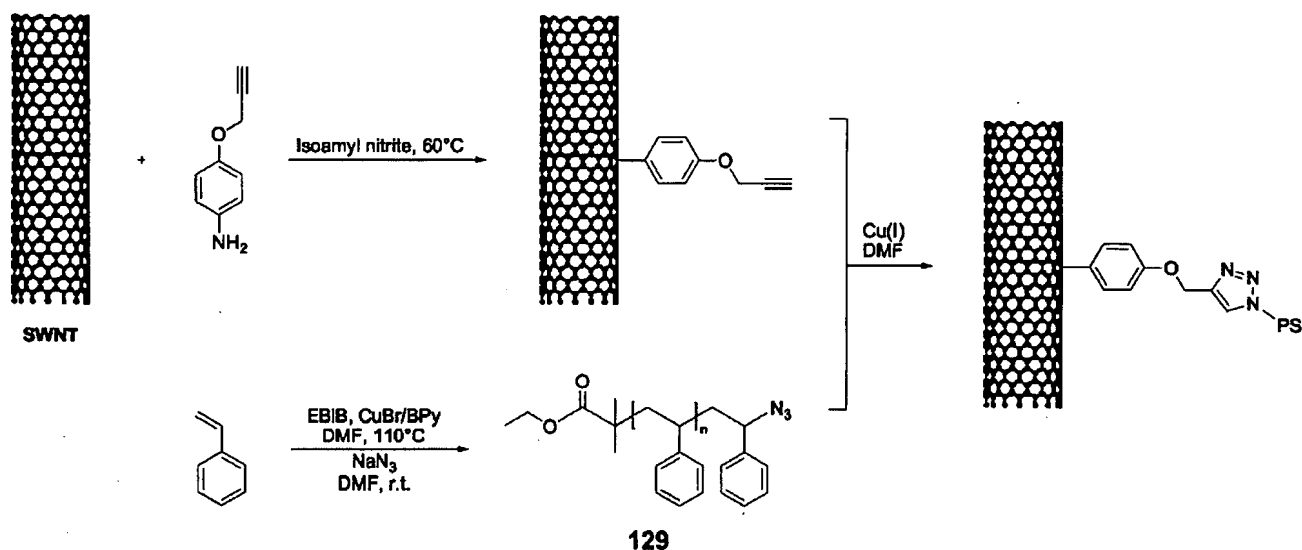h explains the enormous impact this reaction has already had in its infancy. With respect to polymeric substrates, the reaction definitely speeds up the use of polymers with defined functional ligands, positioned either in the main chain or the respective sidechains. Most importantly, control of ligand density within polymers and surfaces is accessible by this simple method, which enables the study of molecular recognition in more detail. Certainly, new and other click-type reactions will emerge in the future, placing strong, hands-on chemical tools in the hands of the preparative material scientist, with the prospect to build matter in a brick-type fashion by simple chemical reactions.

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The Allylic Azide Rearrangement: Achieving Selectivity

Alina K. Feldman, Benoît Colasson, K. Barry Sharpless,* and Valery V. Fokin*

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received January 31, 2005; E-mail: fokin@scripps.edu; sharpless@scripps.edu

Organic azides are most commonly used to introducte an amino group, and in this rather pedestrian role, their existence is barely noticed. Hence, the special reactivity features[1] of the azide functionality, as revealed in cycloadditions and pericyclic reactions, remain underappreciated, even though the latter are probably the most powerful and useful transformations involving azides.

Copper(I)-catalyzed cycloaddition with terminal alkynes, which results in 1,4-disubstituted 1,2,3-triazoles, is among the recent advances in the chemistry of organic azides.[2] The rare chemical orthogonality of the azide and alkyne functionalities (that is, inertness to acidic and basic conditions) has enabled unique applications of this process in chemical biology, organic synthesis, and materials science.[3] Since olefins, too, are stable in most acid/base environments, one expects that the special case of allylic azides might possess the familial reactivity profile, and it does, even though the azide and the olefin groups are engaged in the dynamic [3.3]-sigmatropic equilibration process[4] shown in Scheme 1.

Since this rearrangement generally creates mixtures of the interconverting allylic isomers, it has been viewed as a liability.[5] The goal of our study was to achieve selective capture of one of these isomers. We envisioned that differences in their reactivity patterns could, in concert with their facile interconversion, prove advantageous. Reported here are the results from two model reactions: the Cu(I)-catalyzed azide−alkyne cycloaddition[2] and MCPBA epoxidation of olefins.[6]

Since both steric and electronic effects can influence reactivity of azides and olefins, the following allylic systems were studied in side-by-side experiments: primary vs tertiary, secondary vs tertiary, and primary vs secondary azides. Each of the three classes was represented by two members: the parent aliphatic azides (1, 5 and 9) and their closely related hydroxylated derivatives (3, 7, and 11), selected to investigate the effect of the heteroatom and, in retrospect, the apparent hydrogen-bonding effects between the hydroxyl and the azide groups. As studies progressed, evidence grew that H-bonding effects could significantly modulate the equilibrium "set-points" in these dynamic systems.

Having earlier noted that the Cu(I)-catalyzed triazole synthesis was somewhat sensitive to the steric environment of the azide,[7] we looked for such effects in the selectivity of product capture. Hence, a variety of allylic azides, all engaged in [3.3]-sigmatropy (albeit more or less facile for a specific case), were submitted to the Cu(I)-catalyzed cycloaddition with phenylacetylene as alkyne. The composition of the mixtures of triazole products was determined by ^{1}H and ^{13}C NMR and LC−MS. The results are summarized in Scheme 2.

Excellent selectivity was observed in the primary vs tertiary and secondary vs tertiary azides series (Scheme 2, entries 1 and 2). No products arising from tertiary azides were detected. Equilibration between the tertiary and the *trans*-primary allylic azides is faster than the Cu(I)-catalyzed reaction; however, the interconversion between the tertiary azide with the *cis* form of the primary azide is
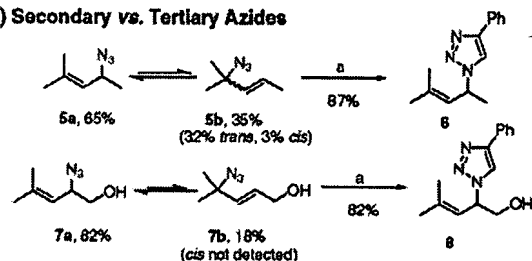
Scheme 1. Rearrangement of Allylic Azides
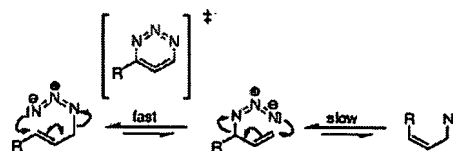
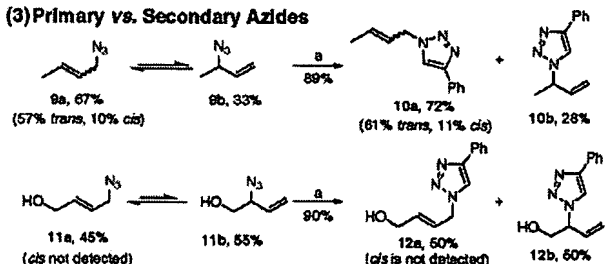**Scheme 2.** Cu(I)-Catalyzed Cycloaddition of Allylic Azides with Phenylacetylene[a]

(1) Primary *vs.* Tertiary Azides
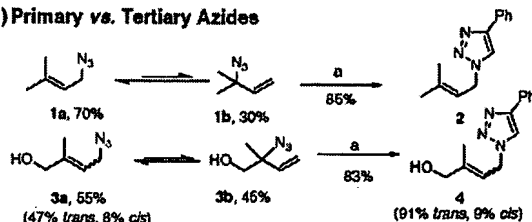
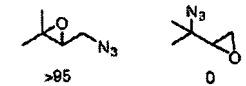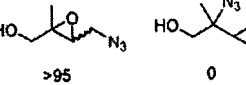(2) Secondary *vs.* Tertiary Azides
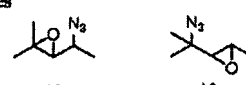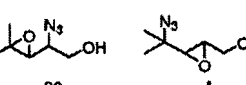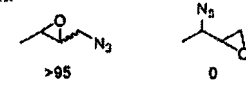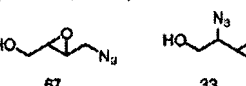

(3) Primary *vs.* Secondary Azides

[a] Reagents and conditions: (a) azide (1 mmol), phenylacetylene (1 mmol), CuSO4·5H2O (0.05 mmol), sodium ascorbate (0.1 mmol), tBuOH/H2O 1:1 (2 mL), room temperature, 12 h.

generally slower.[4c] As a result, the amount of product derived from the *cis* isomer of the azide approximated the amount of the *cis* azide found at equilibrium (as in triazole 4 derived from azide 3a).

Interestingly, the Cu(I)-catalyzed cycloaddition reaction did not distinguish well between the primary and secondary azide regioisomers, with composition of the resulting mixture of triazole products being very similar to that of the starting materials (Scheme 2, entry 3).

To see if differential olefin reactivity would draw out similar selectivity, epoxidation with MCPBA, a reagent well known to

Table 1. Epoxidation of Allylic Azides

allylic azide (regio ratio)	condi-tions[a]	epoxide products (ratio)	yield, (%)[b]
Primary vs. Tertiary Azides			
1	1a:1b (70:30)	A	>95 13a / 0 13b	85
2	3a:3b (55:45)	B	>95 14a (81% trans, 9% cis) / 0 14b	72
Secondary vs. Tertiary Azides			
3	5a:5b (65:35)	A	90 15a / 10 15b	81
4	7a:7b (82:18)	B	96 16a / 4 16b	83
Primary vs. Secondary Azides			
5	9a:9b (67:33)	A	>95 17a (87% trans, 8% cis) / 0 17b	96
6	11a:11b (45:55)	B[c]	67 18a / 33 18b	44

[a] Conditions A:[6b] MCPBA (1.1 equiv), H$_2$O (0.3 M NaHCO$_3$), room temperature, 12 h. Conditions B: MCPBA (1.3 equiv), CH$_2$Cl$_2$, room temperature, 12 h. [b] Isolated combined yields. [c] Reaction time was 48 h.

be sensitive to the electronic properties of the olefin,[6b] was chosen. The epoxide product mixtures were analyzed by ^{1}H NMR, ^{13}C NMR, and GC. Table 1 summarizes the results.

In general, good to excellent selectivity was realized. Aqueous conditions (A), which utilize buffered MCPBA, were preferred (see Supporting Information for details). However, for azido alcohols 3, 7, and 11, nonaqueous conditions (B) were required to achieve complete conversion to the corresponding azidoepoxides. In the primary vs tertiary and primary vs secondary azide systems, excellent selectivity was observed for compounds 1 and 9 (cf. entries 1 and 5). As expected, more substituted olefins reacted faster. The more sluggish rearrangement rates noted with azido alcohols 3 and 11 appear to be due to the interplay of inductive electronic effects[8] and hydrogen bonding effects.[9] Although MCPBA reacts more

slowly with less substituted olefins **3b** and **11b**, they appear to be sufficiently long-lived to produce noticeable amounts of the corresponding azidoepoxides **14b** and **16b**. In the secondary vs tertiary azide series, azido alcohol 7 performed slightly better than azide 5, which lacks a hydroxyl group.

In summary, an allylic azide's existence as a dynamically equilibrating mixture of all possible [3.3]-isomers can be manipulated in interesting ways. By use of an appropriate capture trick, a given [3.3]-rearrangement family of allylic isomers is uniquely "siphoned off" through the isomer preferred by the "fixing" reaction. In the cases at hand, the rearrangement process was terminated by reactions selective for azide functionality and for olefin functionality, respectively. Given the wealth of useful olefin reactions with electrophiles and oxidants, allylic azides appear to offer many worthwhile selectivity refinements, in the already wide world of olefin transformations.

Supporting Information Available: Typical experimental procedures and spectral characterization of all products. This material is available free of charge via the Internet at http://pubs.acs.org.

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FOCUS REVIEWS

Triazole: the Keystone in Glycosylated Molecular Architectures Constructed by a Click Reaction

Alessandro Dondoni*[a]

Dedicated to Professor Rolf Huisgen
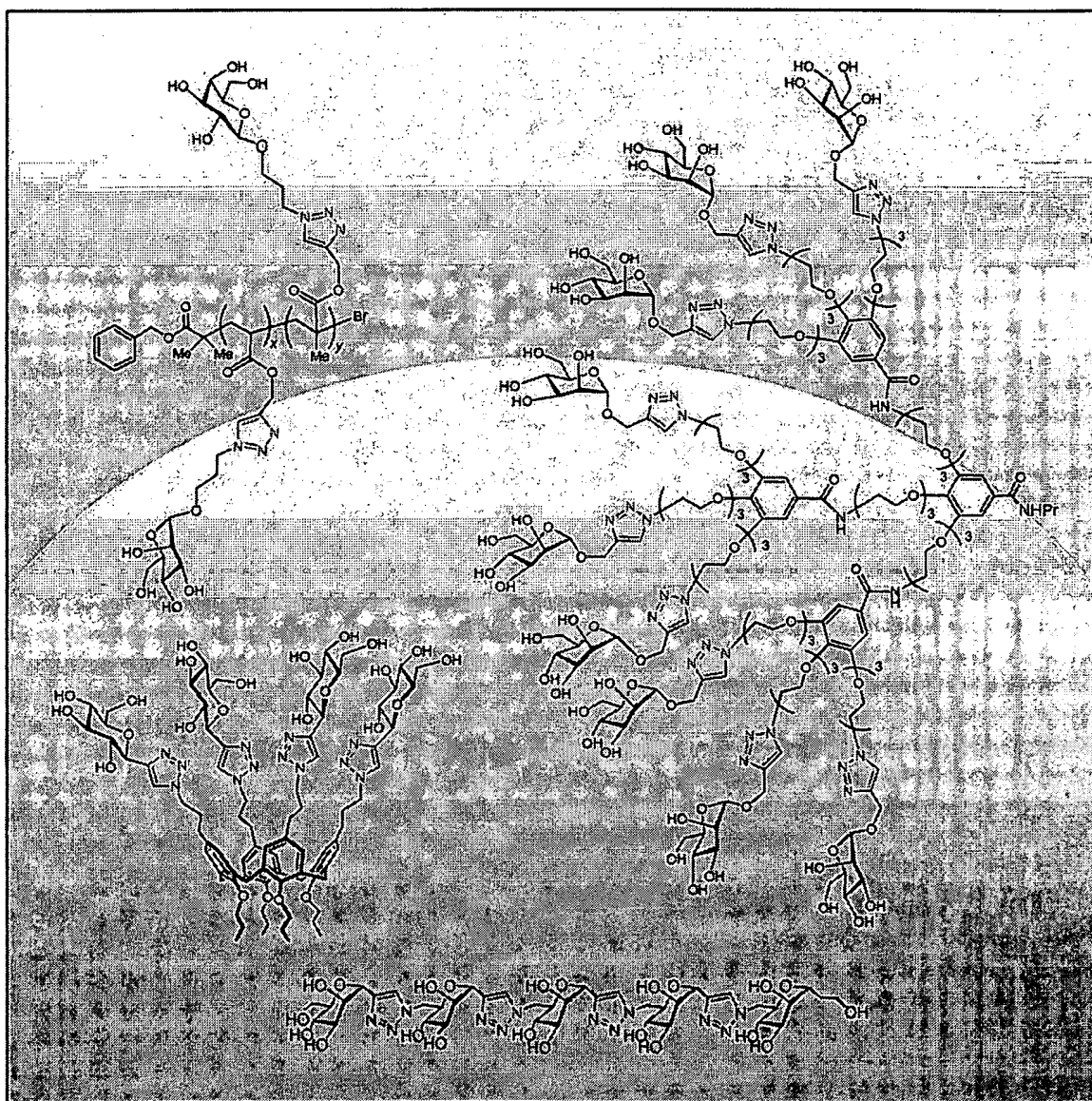
Abstract: The copper(I)-catalyzed modern version of the Huisgen-type azide–alkyne cycloaddition to give a 1,4-disubstituted 1,2,3-triazole unit is introduced as a powerful ligation method for glycoconjugation. Owing to its high chemoselectivity and tolerance of a variety of reaction conditions, this highly atom-economic and efficient coupling reaction is especially useful for the effective construction of complex glycosylated structures such as clusters, dendrimers, polymers, peptides, and macrocycles. In all cases the triazole ring plays a key role by locking into position the various parts of these molecular architectures. The examples reported and briefly discussed in this short review highlight the use of this reaction in carbohydrate chemistry and pave the way to further developments and applications.

Keywords: click reactions · cycloaddition · glycoconjugates · oligosaccharides · triazoles

1. Introduction

Today, organic chemistry, and organic synthesis in particular, is enjoying an exciting period of intense and fertile activity. A large part of research is carried out with a view to application in other fields, mainly biology, medicine, drug discovery, and functional materials. Hence, there is an evolving synergy between advanced organic chemistry and other disciplines with incommensurable advantages on both sides. The great potential of modern organic synthesis is a consequence of recent innovations in reactions, catalysts, and methodologies, as well as the advent of potent analytical techniques. The rediscovery of "old" reactions invented in the middle of the last century has also provided great stimulus to develop new chemistry. Two recent cases are emblematic. One is provided by olefin metathesis, pioneered by Natta, Banks, and Bailey in 1964, of which significance was highlighted by the award of the Nobel Prize to three organic chemists (Chauvin, Schrock, and Grubbs)[1] in 2005. The other is represented by asymmetric synthesis with metal-free chiral organocatalysts,[2] pioneered by two industrial groups (Hajos and Parrish; Eder, Sauer, and Wiechert) in the early 1970s and practically ignored until the 2000s. Both metathesis of unsaturated systems as a wide-scope carbon–carbon bond-forming reaction and organocatalysis as a fundamental synthetic methodology are nowadays privileged processes in organic synthesis. Another reaction that is the fruit of a reevaluation of earlier reported chemistry is copper(I)-catalyzed azide–alkyne coupling to give 1,4-disubstituted 1,2,3-triazole derivatives (Scheme 1). This reaction has garnered a
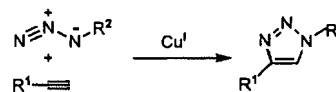
Scheme 1. Cu^I-catalyzed azide–alkyne coupling.

significant amount of attention by researchers operating in disparate fields pertinent to the life and materials sciences.[3] It is amply documented in books and collections[4] that the uncatalyzed thermally induced reaction of organic azides (1,3-dipole) with acetylenes (dipolarophile) belongs to the vast repertoire of [3+2] concerted reactions (one-step pericyclic processes)[5] that have been systematized in the early 1960s by Huisgen under the name of 1,3-dipolar cycloadditions.[6] The discovery that catalytic Cu^I increases the reaction rate and controls the regioselectivity to give the 1,4-disubstituted triazole group while suppressing the formation of the 1,5-regioisomer was made independently in recent times by Sharpless[7] and Meldal[8] and their co-workers. Notably, under the new Cu^I-catalyzed conditions, the reaction does not retain its original concerted nature and proceeds through a multistep mechanism involving azide–copper and alkyne–copper complexes.[9] Hence, the improved version of the Huisgen-type reaction turned out to be a remarkable example of chemical efficiency, because besides being operatively simple and tolerant to atmospheric conditions, it transforms readily the reagents into a single product in high yield and with the maximum level of atom economy. For these reasons, the reaction was identified as the premier ex-

[a] Prof. Dr. A. Dondoni
Dipartimento di Chimica
Laboratorio di Chimica Organica
Università di Ferrara
Via L. Borsari 46, I-44100 Ferrara (Italy)
Fax: (+39)0532-455-167
E-mail: adn@unife.it

FOCUS REVIEWS

ample of a "click reaction", an onomatopoeic expression (click is the sharp sound that is made by snapping one's fingers, for example, to point to a facile and quick operation) coined by Sharpless and co-workers[10] "to indicate a near-perfect chemical process for the assembly of specially designed building blocks". In a similar figurative manner, we consider the triazole unit as a solid keystone[11] that holds separate units together in complex molecular architectures, just as the keystone in an arch secures the whole structure (Figure 1).
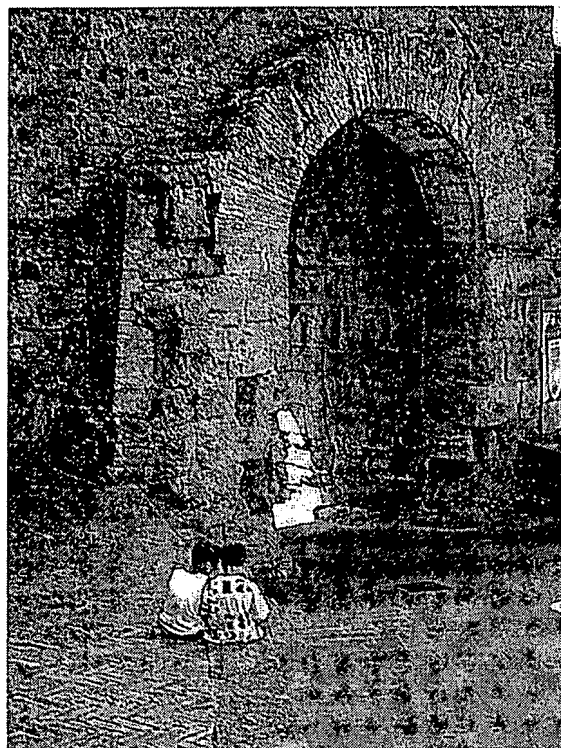

Figure 1. Etruscan arch in the city of Volterra, Tuscany (Italy).

Alessandro Dondoni graduated at the Univ. of Bologna in 1960 under the supervision of Prof. F. Montanari. After a post-doctorate (1961–62) with Prof. S. I. Miller at the Illinois Institute of Technology in Chicago, he returned to the Univ. of Bologna first as Assist. Prof., then as Assoc. Prof. He moved to the Univ. of Ferrara as a Full Prof. of Organic Chemistry in 1975. He has received many awards, such as the Japan Society for the Promotion of Science Award (1988) and the Lincei National Academy Prize in Chemistry (1999). His present research interests include asymmetric and diastereoselective synthesis, heterocycles as synthetic auxiliaries, and carbohydrate chemistry.

The high affinity of the azide[12] for the alkyne group (chemoselectivity) and, by contrast, the inertness of both functionalities toward the majority of functional groups connected to the core of a variety of biomolecules (bioorthogonality), as well as the stability of the triazole ring toward chemical and enzymatic degradation,[13] are all features that concur to make this click reaction particularly suitable for covalently linking bioactive molecular entities, an operation in which mild and neutral conditions are a prerequisite. The potential of this coupling reaction in bioconjugation[14] appears to be comparable to that of the Staudinger reaction between azides and phosphines,[15] a ligation reaction that in a reengineered form was highlighted by Bertozzi and co-workers as a means of selectively modifying cell-surface glycans with exogenous probes.[16] The two methods are complementary. However, the triazole residue produced in the Huisgen-type reaction is more robust than the amidic tether formed in the Staudinger reaction and can participate in hydrogen-bonding and dipole interactions, which can favor the binding to biomolecular targets and improve solubility.[17] Hence, the copper(I)-catalyzed Huisgen-type ligation did not escape the attention of synthetic carbohydrate chemists in their continuous efforts to devise new tools that might allow molecular glycobiologists to clarify the role of carbohydrates at the molecular or cellular levels in biological events.[18] Alternatively, these tools can be advanced structures for the developments of new therapeutics[19] or carbohydrate-based vaccines[20] against largely diffuse human diseases such as cancer, inflammation, and viral infections. Herein, I report and comment on some triazole-based glycosylated structures whose construction relies on the readiness and efficiency of the copper(I)–azide–alkyne-coupling (CAAC) process. The presence of carbohydrate systems with their delicate structure rich in functionalities and stereocenters makes these structures one of the most severe testing benches on which to validate the utility of this reaction. Although this account is not meant to be a comprehensive overview, the achievements from different laboratories that bear substantial witness to the versatility of the CAAC reaction will be illustrated.

## 2. Early Applications: Triazole-Based Glycosylated Arrays and Glycoclusters

Wong[21] and Santoyo-Gonzáles[22] and their co-workers were timely in reporting in 2002, the same year of the discovery of the Cu[I] catalysis,[7,8] the efficient ligation of carbohydrates with non-carbohydrate substrates by the CAAC process. Wong and co-workers[21] described the construction of glycosylated arrays on the surface of microtiter wells (Scheme 2). Quite remarkably, they not only exploited the azide–alkyne ligation to attach oligosaccharides to a hydrocarbon chain that was not covalently bound to the microtiter plate, but also showed that the glycosidic portion could be modified, for example, fucosylated, by an enzymatic reaction. Various biological assays were also carried out, thus demonstrating
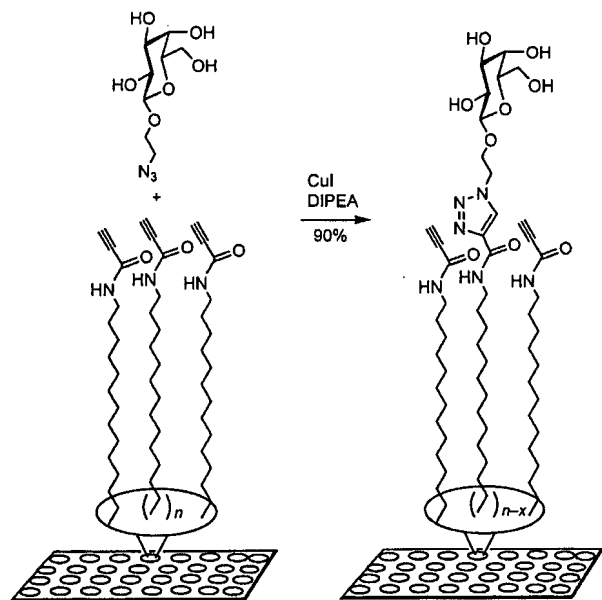
Scheme 2. Formation of a glycosylated array in a microtiter plate by the CAAC process.[21] DIPEA = diisopropylethylamine.

that the carbohydrate arrays were suitable for high-throughput studies of protein interaction and enzyme-inhibitor screening. The road was thus paved for the synthesis of other carbohydrate arrays by the CAAC process.[23] In all cases, the presence of the triazole ring, with its impervious nature to degradation by external agents, was crucial to robust ligation between the glycosylated fragment and the molecular unit immobilized on the solid surface.

Santoyo-Gonzáles and co-workers[22] prepared a range of glycoclusters anchored to various scaffolds. An example of these compounds is shown in Scheme 3. This pioneering work was also important in many respects as it demonstrated the fidelity of CAAC chemistry in a program directed toward the introduction of several sugar fragments in a single molecular residue.
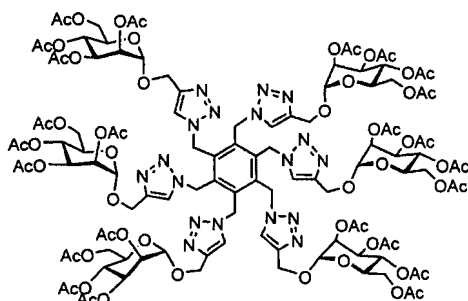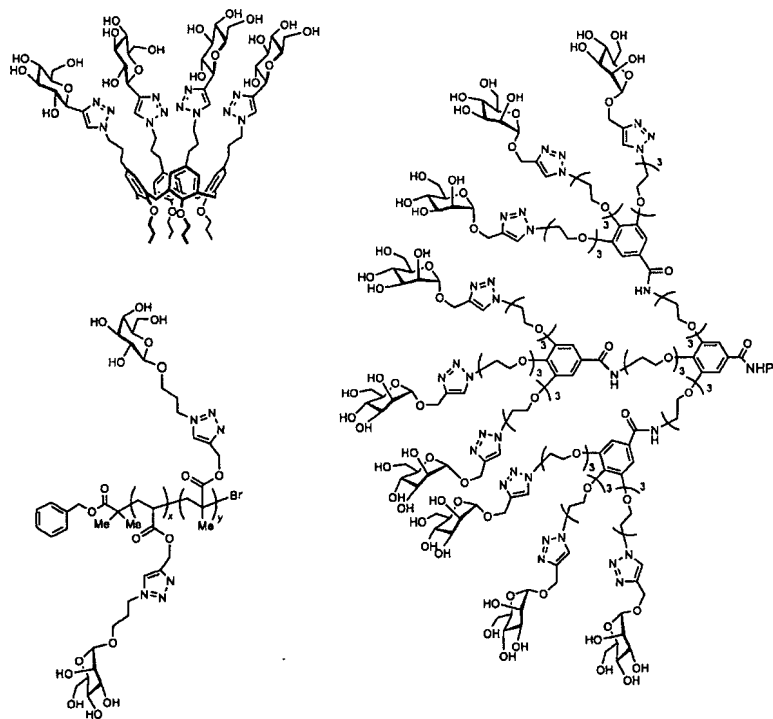
# 3. Other Triazole-Based Glycoclusters, Glycodendrimers, and Glycopolymers

The synthesis of multivalent neoglycoconjugates is currently promoted by the extensive findings of multiple ligand–receptor interactions that occur in nature and by the phenomenon generally referred to as the glycoside cluster effect.[24,25] A number of papers have appeared in 2005 and 2006 that deal with the CAAC-based synthesis of various types of densely glycosylated molecular architectures, such as glycoclusters,[26] glycodendrimers,[27] and glycopolymers[28] (Scheme 4). The preparation of these saccharide constructs involved the equipping of a suitable scaffold with several azide or ethynyl groups and then treatment with a glycosylated residue holding the complementary functionality for performing concomitant copper(I)-catalyzed Huisgen-type reactions. From the numerous substrate combinations employed and the high yields of the multivalent glycoconjugates obtained, it appears that each CAAC reaction proceeded quite efficiently regardless of the structure and complexity of the substrate to which the azide and ethynyl groups were bound. Surprisingly, however, there was no apparent concern about the stability of compounds displaying an O- or N-glycosidic bond to connect the carbohydrate moiety, either directly or through a suitable spacer, to the triazole ring. It is a common tactic in glycochemistry to replace the O- and N-glycosidic residues with C-[29] or S-glycosidic analogues[30] to induce high stability toward chemical and enzymatic degradation without compromising biological activity. In accordance with this concept, this structural feature was introduced in the calix[4]arene-based C-glycocluster shown in Scheme 4 and in other similar products reported in the same publication from our laboratory.[26c] This improvement should avoid serious drawbacks such as the easy removal of the carbohydrate fragments from glycoclusters when they are employed, for example, as probes in glycobiology or biosensors in nanodevices under conditions that are not tolerated by the weak O- and N-glycosidic bonds.

4. Triazole-Tethered Glycosyl Amino Acids and Peptides

Another issue that was successfully addressed by CAAC chemistry was the synthesis of nonnatural glycopeptides. Extensive findings demonstrate that anomerically O- and N-linked carbohydrate residues play a key role in the folding and biological activity of glycopeptides.[23a,31] Consequently, chemically and metabolically stable analogues composed of C-glycosyl α-amino acids are important synthetic targets because they can serve as probes in studies of biochemical pathways and leads for the development of potential drugs.[32] Hence, the synthesis of a new class of C-glycosyl α-amino acids that contain the triazole ring as a linker of the sugar fragment and the amino acid moiety (Scheme 5) was reported independently by us and Rutjes and co-workers.[33] Interestingly, the comparison of the thermal and copper(I)-

Scheme 3. Glycocluster prepared from hexakis(azidomethylbenzene) and propargyl O-mannoside.[22]

Scheme 4. Examples of triazole-linked multivalent neoglycoconjugates: glycocluster[26c] from tetrakis(azidopropylcalix[4]arene) and ethynyl C-glucoside (left top), glycodendrimer[27b] from polyazidogallic acid–triethylene glycol dendrimer and propargyl O-mannoside (right), and glycopolymer[28] from a copolymer with alkyne side chains and azidopropyl O-glycoside (left bottom).
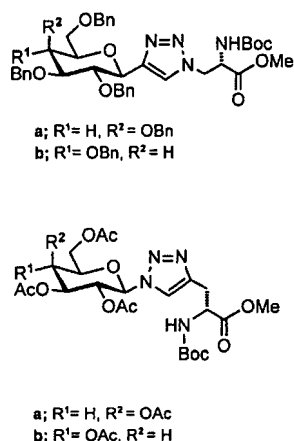
Scheme 5. C-glycosyl[33a] and N-glycosyl[33b] triazolylalanines (top and bottom, respectively) prepared by the CAAC process. BOC=tert-butoxycarbonyl.

catalyzed reactions between ethynyl C-glycosides and azide-equipped amino esters[33a] demonstrated the total 1,4-regioselectivity of the latter process,[34] whereas the uncatalyzed reaction afforded the two 1,4- and 1,5-triazole regioisomers in comparable amounts. More extensive work by Rutjes and co-workers[33b] was carried out in the synthesis of triazolyl

amino acids and peptides, all of which display the glycosyl fragment bound to a nitrogen atom of the heterocyclic ring.

Very recently, carbohydrate-decorated polypeptides (Scheme 6) constructed by the CAAC process were reported by Danishefsky and co-workers.[35] This is an important and notable contribution to the ongoing research on the synthesis of carbohydrate-based antitumor vaccines. These substances could either be conjugate systems composed of oligosaccharides covalently linked to immunogenic proteins or simply polysaccharide antigens.[20] These systems could induce an immune response because cancer cells typically display aberrant levels and patterns of cell-surface glycosylation.[20a] The strategy employed by Danishefsky and co-workers involved the introduction of the azide group at the end of a glycopeptide chain followed by carry-on multiple copper(I)-catalyzed couplings of this substrate with a polypeptide equipped with pendant alkynyl groups. However, in contrast to that observed in simple model systems, the construction of such large architectures from highly sensitive substrates revealed that the key CAAC process needed optimized conditions. The reaction time was thus reduced from days to hours, and the yields of the desired adducts were increased from low to excellent values by the use of nanosized Cu particles in slightly basic water.

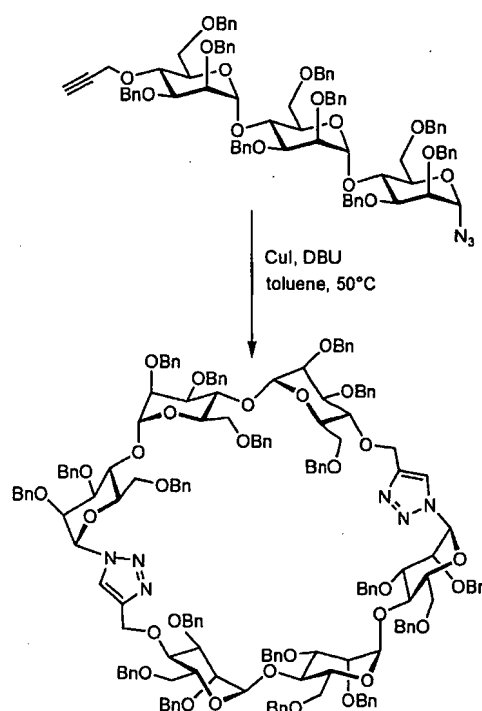## 5. Triazole-Carbohydrate Hybrid Macrocycles

Recent papers provided evidence on the utility of the CAAC process in the synthesis of cyclic oligomers. Inspired by the general tactic of Fürstner and Müller in cyclic glycolipid synthesis by ring-closing metathesis,[36] Dörner and Westermann reported the preparation of triazole-tethered carbohydrate macrocycles.[37] Dimeric carbohydrate–triazole fragments bearing a carbon–carbon double bond at each end were first prepared by the CAAC process. These intermediates were then subjected to macrocyclization via intramolecular olefin metathesis promoted by the first-generation Grubbs ruthenium–carbene complex. Besides paving the way to libraries of carbohydrate–heterocycle hybrid macrocycles, this work furnished an important result as it
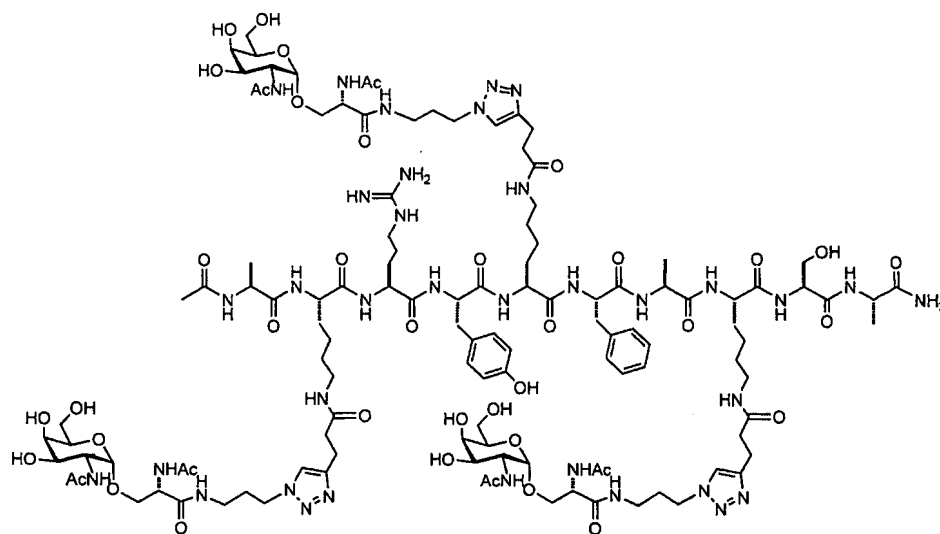
Scheme 6. Example of polypeptide with three side chains bearing a triazole ring and a terminal O-glucoside residue.[35]

demonstrated the compatibility of the triazole ring with the conditions of olefin metathesis. This finding can be of great utility in the design of new synthetic strategies and projects.

A highly convergent approach based exclusively on CAAC chemistry was employed by Gin and co-workers in the preparation of cyclodextrin analogues that display two or more triazole residues as part of the ring.[38] A notable example is provided by the synthesis of an oligosaccharide macrocycle that involved the cyclodimerization of a trisaccharide carrying an azide group at one terminal and an eth-ynyl group at the other (Scheme 7). The reaction was effectively promoted by CuI and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in toluene. On the other hand, in the absence of the copper catalyst, the reaction was very sluggish and afforded the target oligosaccharide in small amounts together with multiple products. The same synthetic route was followed by Billing and Nilsson for the cyclooligomerization of bifunctional (azide and ethynyl) monosaccharide–dipetide substrates to give macrocyclic carbohydrate–dipeptide hybrids.[39] In this case, the most efficient conditions were found by the use of CuI and N,N-diisopropylethylamine in MeCN. Hence, this and the other cases illustrated above indicate that despite its simplicity, the CAAC reaction applied to multifunctionalized systems require optimized conditions especially with respect to the generation of the copper(I) catalyst.
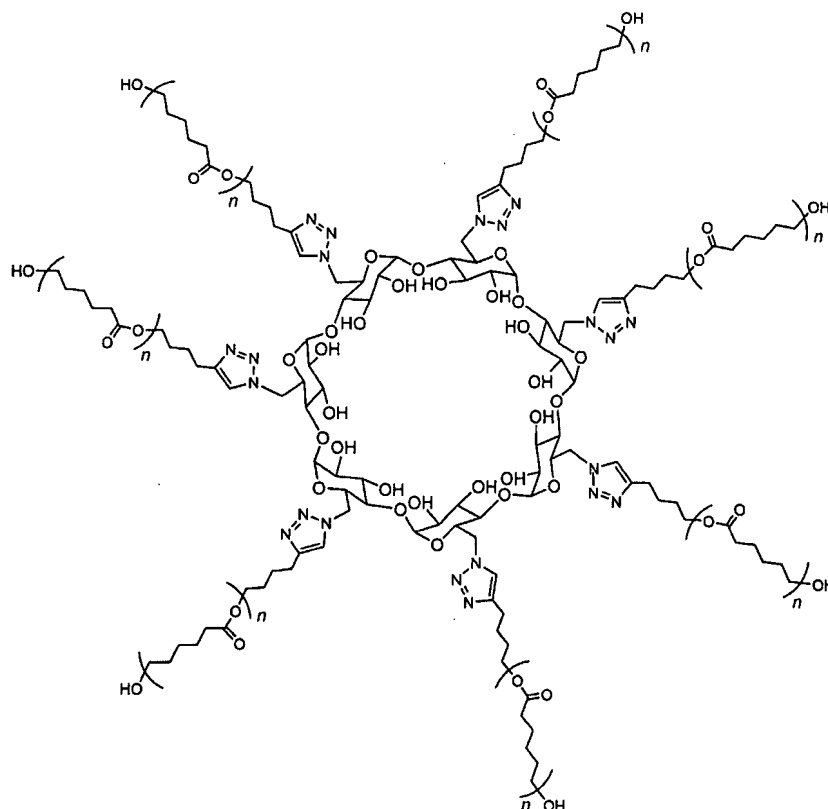
6. Cyclodextrin with Triazole-Tethered Polymer Chains

The enduring interest of many researchers for cyclodextrin functionalization led to the application of the CAAC process to this natural macrocyclic oligosaccharide as well.[40] Hence, β-cyclodextrin functionalized with seven azide

Scheme 7. Synthesis of a triazole-linked oligosaccharide macrocycle by the CAAC process.[38]
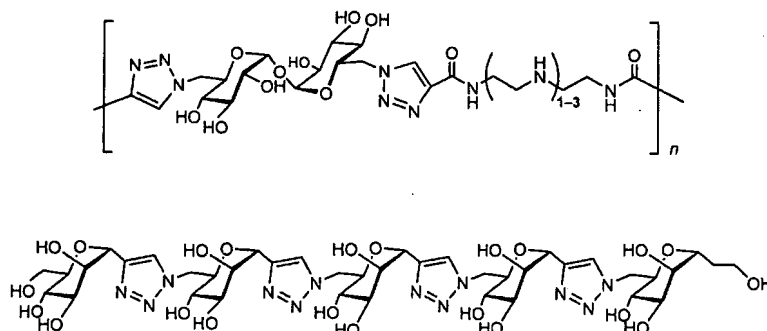
groups, one for each glucose residue, and an excess of acetylene poly(ε-caprolactone) under CuI catalysis and microwave acceleration afforded the star-shaped macromolecule shown in Scheme 8.

FOCUS REVIEWS

Scheme 8. Heptakis(poly(ε-caprolactone)-β-cyclodextrin) with triazole tethers.[40]

7. Glycopolymers with Triazole Rings in the Chain

Open-chain glycopolymers with incorporated triazole units were constructed by Reineke and co-workers[41] in early 2006 (Scheme 9). The key operation for polymer formation consisted of multiple Cu^I-catalyzed coupling reactions (click polymerization) between trehalose bis(azide) and dialkyne oligoamine monomers. Three polymers were constructed by varying the amine number between the trehalose moieties.
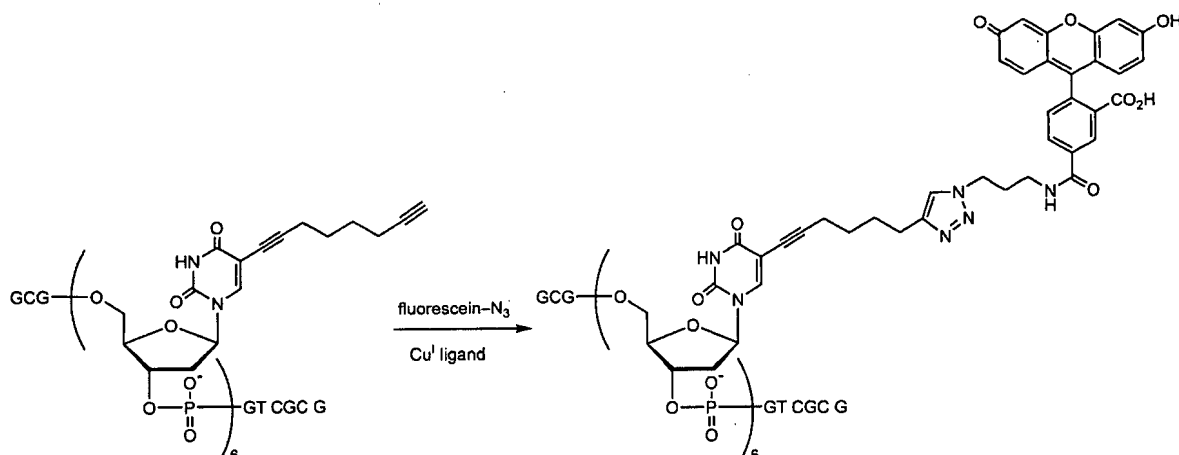
The synthesis of these compounds represents another remarkable success of the CAAC process in the preparation of complex glycosylated architectures with planned biological activity. In fact, the glycopolymers were synthesized to promote nucleic acid delivery into cells in the presence of serum. Therefore, the polymeric structures were designed with a special role for each constituent in mind. In particular, the heterocycle–amide groups were thought to promote complexation with nucleic acids through hydrophobic, van der Waals, and H-bonding interactions. For similar reasons we prepared the triazole-linked oligosaccharide triazolopentamannose[42] (Scheme 9). This new type of carbohydrate–heterocycle hybrid, which displays only C-glycosidic linkages, represents an anomerically stable imitation of natural mannooligosaccharides that constitute the essential substructure of mycobacteria lipoglycans. Hence, these artificial oligosaccharides may act as inhibitors of the growth of these organisms, which are the cause of diseases such as tuberculosis.[43] The triazole–mannose oligomer was prepared by four repetitions of a reaction sequence comprising the CAAC process under microwave irradiation and azidation of the resulting product. This iterative process appears to be perfectly suited to the preparation of sugar oligomers with a well-defined composition. For simplicity as well as chromatography-free and high-yield reactions in each cycle, this reaction is likely to be carried out in an automated apparatus.

8. Labeling of DNA Strands

A postsynthetic method was employed for the introduction of molecular labels in DNA strands by CAAC chemistry.[44] The method was first applied to designed alkyne-modified oligodeoxyribonucleotides, which were subjected to multiple coupling with suitable azide-bearing labels such as glucose, cou-

Scheme 9. Triazole-containing glycopolymer[41] (top) and oligosaccharide[42] (bottom) constructed by the iterative CAAC process.

Scheme 10. Synthesis of fluorescein-labeled oligodeoxyribonucleotide by multiple CAAC processes.[44]

marin, and fluoroscein (Scheme 10). As strand breaks occurred in the presence of Cu^I, this inconvenience was avoided by the use of the Cu^I-stabilizing ligand tris(benzyltriazolylmethyl)amine, which is known to protect biomolecules from side reactions in water that involve Cu^I ions. The CAAC reaction also proved effective with alkyne-containing long DNA fragments obtained by enzymatic processes such as the polymerase chain reaction (PCR) without the DNA cleavage. This postsynthetic modification was introduced as a means of allowing the decoration of DNA for isolation and identification according to the nature of the probe.

9. Conclusions

In summary, the Huisgen-type azide–alkyne cycloaddition promoted by copper(I) is a powerful tool in new strategies for complex glycoconjugate synthesis. The reaction has been employed especially in the preparation of glycosylated substrates for studies of carbohydrate-based molecular-recognition processes and for advancement in the synthesis of designed glycoconjugates to prevent diseases of social relevance such as cancer. In all cases in which the reaction was interrogated, it gave a positive response and showed its superior value relative to the thermally induced process. Concomitant multiple cycloadditions allowed the decoration of complex scaffolds such as dendrimers and polymers with numerous carbohydrate residues. Repetition of the reaction in oligosaccharide synthesis by a linear-homologation strategy showed unvarying efficiency over several consecutive cycles. The role of the triazole group as a robust keystone that firmly holds disparate types of molecular fragments emerged in all circumstances. Finally, although the main contribution of this reaction is mainly manifested in the realm of organic synthesis, the skepticism about its application in living systems owing to the noxious copper catalyst is being dissolved by the successful live-cell labeling carried out by Link and Tirrell[45] as well as Bertozzi and co-workers.[46]

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The Rise of Azide–Alkyne 1,3-Dipolar 'Click' Cycloaddition and its Application to Polymer Science and Surface Modification

Richard A. Evans[A,B]

[A]CSIRO Molecular and Health Technologies, Bag 10, Clayton South VIC 3169, Australia.
  Email: richard.evans@csiro.au
[B]Centre for Advanced Macromolecular Design (CAMD), School of Chemical Engineering and
  Industrial Chemistry, University of New South Wales, Sydney NSW 2052, Australia.

New methods to synthesize and functionalize polymers are of constant interest to the polymer scientist. The 1,3-dipolar cycloaddition between an azide and terminal alkyne has received much attention since the reports that copper(I) provides high yields and regioselective synthesis of 1,4-substituted 1,2,3-triazoles. This coupling chemistry has been rapidly adopted by polymer scientists in the synthesis and post-polymerization modification of polymers. This Review will provide the historical context of the recent development of the copper-mediated azide–alkyne cycloaddition and its use in polymer science, particularly in dendrimer synthesis/functionalization, surface immobilization/modification, orthogonally functionalizing polymers, and its integration with ATRP (atom transfer radical polymerization).

Introduction

The term 'click' chemistry was originally coined in a generic sense to mean reactions that are easy to perform and work up, high yielding, and tolerant of oxygen or water.[1,2] However, there has arisen one particular reaction that has become the de facto leader of the field and that is the Huisgen 1,3-dipolar cycloaddition of terminal alkynes with azides to give 1,2,3-triazoles. It was the near simultaneous reporting[3,4] of the use of copper(I) to make the reaction faster and regio-specific by two research groups that has made the cycloaddition so prominent. Originally, an initial focus of the reaction was bio-conjugation[3,5] because of the mild reaction conditions, but it has been rapidly appropriated by major polymer groups. This review will outline the synthetic basics of 1,3-dipolar cycloaddition chemistry and how it has been applied to polymer science.

1,3-Dipolar 'Click' Cycloaddition Basics

The Huisgen 1,3-dipolar cycloaddition considered here is the reaction of a terminal acetylene (a dipolarophile) with an azide (a 1,3-dipole) to generate a 1,2,3-triazole (Fig. 1). The '1,3' refers to the atoms of the dipole that undergo a reaction whereas the [3+2] designation that can be occasionally seen[5] refers to the
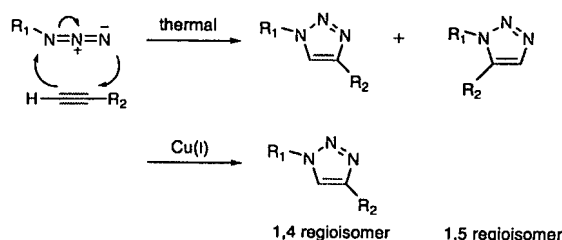

Fig. 1. The 1,3-cycloaddition of an azide and terminal alkyne to give a 1,2,3-triazole. Thermal cycloaddition is non-regiospecific whereas the copper(I)-mediated reaction is 1,4-regioselective.[3,4] The movements of the electron pairs are added for clarity only and are not meant to imply concertedness or non-concertedness of the reaction.

number of members provided by each reactant to the new ring. The many other types of 1,3-dipolar cycloadditions will not be covered by this review.[6,7]

Life Before Copper

Prior to the use of copper(I), the cycloaddition of azides with terminal acetylenes required elevated temperatures for prolonged

Richard Evans is a Principal Research Scientist at CSIRO Molecular and Health Technologies and is an Adjunct Professor at both the Centre for Advanced Macromolecular Design (CAMD) School of Chemical Engineering and Industrial Chemistry, University of New South Wales, and the Centre for Micro-Photonics, Faculty of Engineering and Industrial Sciences, Swinburne University of Technology. His research interest is in the design, synthesis, and application of functional polymers. He has published/patented in the areas of photochromism, free radical polymerization, optical data storage, organic reactive intermediates, and prebiotic chemistry.
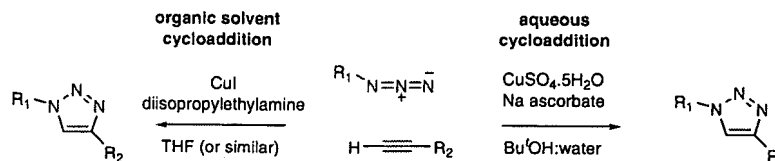
organic solvent
cycloaddition

aqueous
cycloaddition

$R_1-N\diagdown N\diagdown N$ ← Cul
diisopropylethylamine
THF (or similar)

$R_1-N=N=\bar{N}$
$\overset{+}{}$

$H\equiv R_2$

CuSO$_4$.5H$_2$O
Na ascorbate
→
Bu^{t}OH:water

$R_1-N\diagdown N\diagdown N$
R_2

Fig. 2. The two major implementations of copper(I)-mediated azide–alkyne 1,3-cycloaddition. Organic-solvent-based cycloaddition uses copper(I) directly with an amine and protection from oxygen,[3] and aqueous cycloaddition uses ascorbate to generate copper(I) in situ from copper(II) sulfate and is less sensitive to oxygen.[4]

periods.[8] Typically, conditions involved refluxing toluene or carbon tetrachloride for 10–48 h. The cycloaddition was non-regiospecific with two possible isomers (1,4 and 1,5) being formed (Fig. 1). Some control of regiospecificity is obtainable as electron-withdrawing groups on the acetylene favours production of 1,4 products, and electron-withdrawing groups on the azide favours production of the 1,5 isomer. In practice, mixtures were still obtained and exclusive production of one isomer by cycloaddition proved elusive.[8] Thus, the reaction in this form was not suitable for the requirements of a 'click' reaction.

The Copper(I) Revolution

The turning point for the Huisgen 1,3-dipolar cycloaddition came with the discovery that copper(I) not only promotes the speed of the reaction but also the regiospecificity with exclusive production of the 1,4-triazole isomer (Fig. 2). The first publication was by Meldal et al. (in 2002), which outlined the use of copper(I) in the cycloaddition reaction for triazole synthesis on a solid phase.[3] This was an organic-solvent-based procedure that used copper(I) iodide with N,N-diisopropylethylamine (DIPEA) in various solvents with the terminal alkyne immobilized on a swollen solid support. This was closely followed by the report of Fokin and Sharpless et al. that the reaction could be water based using copper sulfate and sodium ascorbate.[4] Both methods have found great popularity for several reasons described below.

Typical Reaction Conditions

Aqueous Cycloaddition

The reaction is extremely straightforward. The azide and terminal alkyne are mixed in a mixture of tert-butanol and water (1:1 or 2:1 etc.), the sodium ascorbate (5–10 mol-%, frequently 10 mol-%) is added and then a copper(II) sulfate pentahydrate solution (1–5 mol-%, frequently 5 mol-%) is added and the flask sealed and vigorously stirred at room temperature.[4] In small molecule chemistry, the method is very forgiving of reaction conditions. The copper(I) is generated in situ by reduction of the copper(II) with an excess of sodium ascorbate and under these conditions the normally oxygen sensitive copper(I) survives happily with simple stoppering of the reaction flask. The small molecule 1,2,3-triazole products are often relatively insoluble in the reaction medium and this results in their precipitation. This makes isolation easy and further purification frequently unnecessary.[4] The reactions are typically run overnight although mild heating shortens reaction time.[9] Aqueous systems can also use copper(I) species directly such that a reducing agent is not needed. The reaction conditions then usually require acetonitrile as a co-solvent (although not essential[10]) and a nitrogen base (like triethylamine, 2,6-lutidine, pyridine, or diisopropylethylamine). In this case the copper(I) species is supplied as CuI, CuOTf·C$_6$H$_6$ or [Cu(CH$_3$CN)$_4$][PF$_6$].[4] Residual copper can be removed by an ammonium hydroxide/citrate buffer,

dithiocarbamate, or by washing with ethylenediamine tetraacetic acid (EDTA) solution.[11–13] The actual reaction mechanism is still under investigation and appears quite complex.[4,14] Essentially, the reaction is non-concerted and is mediated by the copper acetylide. A recent analysis suggests that both azide and alkyne are activated by copper possibly within a multinuclear copper-acetylide species[14] and this follows on from an earlier report of two copper centres participating in the catalysis.[15]

Solvent Cycloaddition

Although the water-based method is attractive for many applications, solvent-based procedures have found utility in situations when solubility problems arise. Copper(I) is supplied directly to the reaction in the form of CuI with N,N-diisopropylethylamine and when co-solvents such as acetonitrile, dichloromethane, tetrahydrofuran (THF), toluene, and N,N-dimethylformamide (DMF) are used. THF with Cu(PPh$_3$)Br and N,N-diisopropylethylamine,[16,17] and CuBr in DMF with bipyridine, are alternate protocols.[18] Many of the reports of azide–alkyne cycloadditions in a polymer context use organic solvent protocols.[11,12,16,18–27]

The Source of Azides

There are a variety of methods[28] to access the azide functionality required of 1,3-dipolar cycloaddition and only the most direct routes will be discussed here. However, it must be noted that the need for azides can be a drawback of the reaction. Sodium azide has a similar toxicity to sodium cyanide.[28] Azides are highly energetic materials and potentially explosive. This is particularly so of low-molecular-weight azides. The convention in working with azides is that the ratio of nitrogen to (carbon + oxygen) should be at most 1:3 for neat isolation of the azide (i.e., nonyl azide is the smallest azide that should be isolated in the neat state under this rule). In practice, ratios slightly richer in nitrogen can be isolated neat. However, compounds with ratios of 1:2 and lower should be viewed and handled with extreme caution. Other sources of risks are the use of metals (i.e., copper) and halogenated solvents (i.e., dichloromethane) while using sodium azide. The popularity of the azide–alkyne cycloaddition has made the knowledge of working safely with azides imperative and the reader is directed to a detailed recent review.[28]

Aliphatic azides can be made through the nucleophilic displacement of a halogen with sodium azide (Fig. 3). The reaction can be carried out in water with sodium azide in a biphasic solution with the product azide easily isolated directly or by extraction.[16] A little sodium iodide may also be used to facilitate the reaction. The traditional method uses DMF[28] as the solvent, although dimethyl sulfoxide (DMSO)[29,30] is an excellent alternative. Aromatic azides can be made by two methods.[31] Aryl hydrazines are easily converted into azides with nitrous acid generated in situ with acid and sodium nitrite.[28,32] Alternatively,
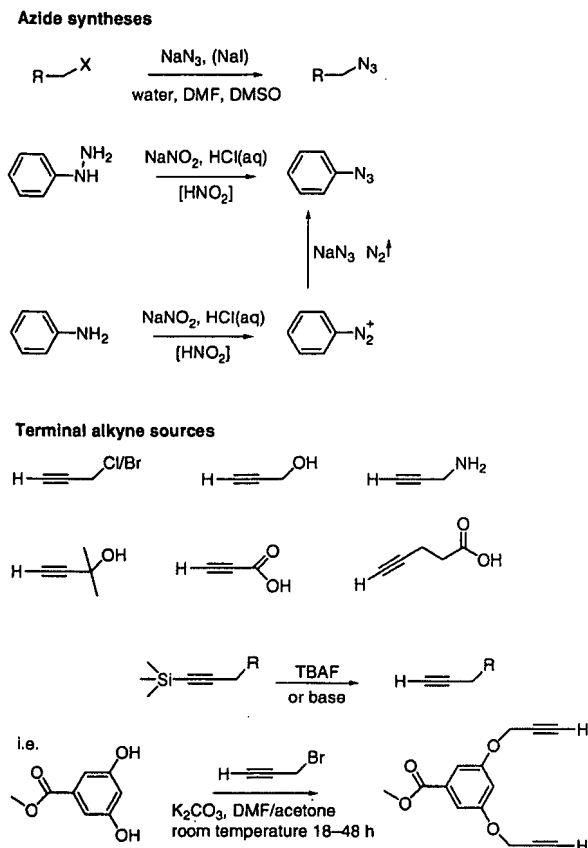
Azide syntheses

Terminal alkyne sources

Fig. 3. Common methods of azide preparation and convenient sources of terminal alkynes.

aryl amines are diazotized with nitrous acid followed by sodium azide to give the azide.[33,34]

The Source of Alkynes

Without doubt the propargyl group (H–C≡C–CH$_2$–) provides the easiest access to functional terminal alkynes through the use of propargyl amine, alcohol, or halide. A common reaction is the propargylation of phenols with either propargyl chloride/bromide[9,16,35,36] or propargyl alcohol with potassium t-butoxide.[37] Second to the propargyl groups is propiolic acid (H–C≡C–COOH)[3] and 4-pentynoic acid derivatives (Fig. 3).[19,38] Naked terminal alkynes appear to have some degree of participation in radical polymerizations. This has been overcome by using the trimethylsilyl alkyne derivative followed by conversion into the terminal acetylene with basic conditions or tetrabutylammonium fluoride.[22,39–42]

Alternate or Modified Procedures

The strong interest in the use of azide–alkyne 1,3-cycloaddition has also resulted in attention given to the basic reaction itself. Although there are many papers that look at variations on the reaction, there are a few that should be mentioned. The brand name of 'click' suggests a degree of speed, however the reaction, although a vast improvement over non-copper solution-based cycloaddition, can appear rather slow with overnight reaction times at room temperatures frequently used.[3,4,10,16]
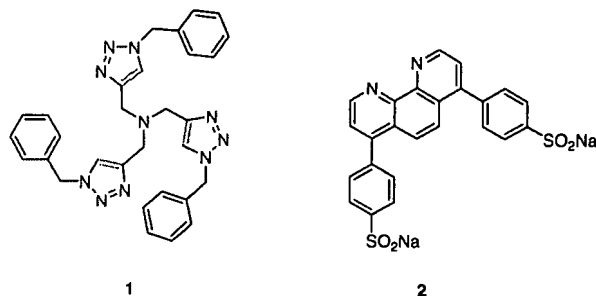

Fig. 4. Ligands that catalyze azide–alkyne 1,3-cycloadditions. Tris(benzyltriazolyl)amine **1** and bathophenanthrolinedisulfonic acid **2**.[10,15]

Additional research has revealed some variants to the method to obtain faster reaction times. Heating can accelerate the reaction although this may not be suitable when biological conjugation is desired.[9] Surprisingly, the use of dichloromethane/water appears to give faster reaction times (approx. 2 h) with equal or higher yields[43] and this protocol may prove useful to polymer scientists whose polymers can often be dichloromethane soluble. Currently, many polymer syntheses or modifications not involving water-soluble materials are performed under organic solvent click conditions.[11,12,16,18–27] The obvious compromise in using dichloromethane is that in small molecule synthesis the product will probably not precipitate from the reaction mixture. This may not be a problem in the reactions of polymers because of ease of purification from small molecules by precipitation of the polymer into methanol or hexane. Another protocol that might prove to be of use in acidic reaction media is that of Orgueira et al., which showed that nanometer-sized copper(0) (10 mol-%) with amine hydrochloride salts (1 equivalent) will give high yields in Bu^{t}OH/water in 2 h.[44] When copper was reduced to 1 mol-%, the reaction required longer reaction times. This work drew upon a footnote (no. 13) in the original Fokin and Sharpless' et al. report that copper metal can also catalyze the reaction.[4] Microwave-assisted synthesis can dramatically accelerate the method. The protocol has two forms. One is the standard organic solvent soluble copper(I) complex simply being heated with microwaves for approx. 20 min.[16] The other generates the azide in situ from an alkyl bromide that reacts with sodium azide, copper metal, and copper sulfate in Bu^{t}OH/water.[45] Reaction times are in the order of 10–15 min with high yields. In this procedure care must be taken that any copper azides (shock sensitive explosives) that may be formed are removed with basic ammonium citrate or dilute HCl.[45]

The Use of Ligands

Poly(1,2,3-triazoles) have been found to be good copper(I) stabilizing/protecting ligands for the 1,3-dipolar cycloaddition reaction.[10] Thus, some syntheses appear self catalytic with the triazole product stabilizing the copper(I). In particular, the ligand tris-(benzyltriazolyl)amine **1** (the product of benzyl azide with tripropargyl amine) is a preferred example. A methodical high throughput study of ligands showed that the best ligands are the tris-triazoles and electron-rich bipyridines. Particularly high reactivity was obtained by the water-soluble bathophenanthrolinedisulfonic acid **2** (Fig. 4).[15] However, the increased oxygen sensitivity of the reaction using that ligand may temper its widespread use.[15] Optimal performance is achieved
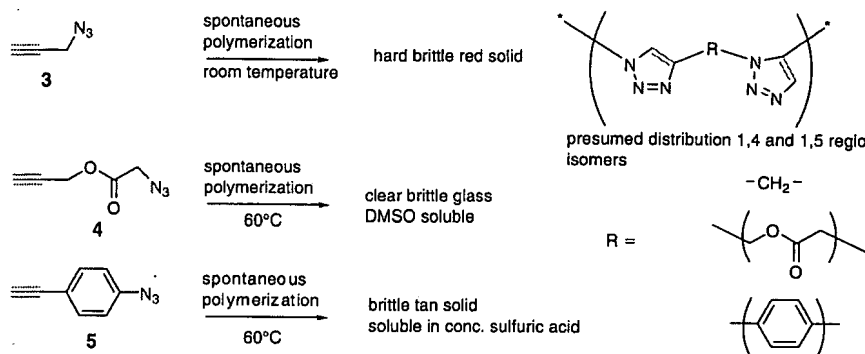
Fig. 5. The 'pre-copper(i)' step-growth 1,3-cycloaddition polymerization of azidoalkynes 3–5. Spontaneous polymerization under mild conditions.[46]

in neutral to slightly basic conditions. There appears to be no example of ligands being used in current polymer work in this manner in aqueous systems. A possible reason may be the risk of contamination of the product with the polytriazole ligand. However, as will be discussed below, they may play a useful role in polymer–biomolecule conjugations and surface immobilization applications.

Current Directions in Polymer Synthesis and Modification

From the above it can be seen that the copper mediated azide–alkyne cycloaddition reaction is a coupling reaction like an esterification, etherification, urethane formation, etc. It joins two moieties together under mild conditions. The azide and alkyne groups are otherwise reasonably inert, meaning a wide range of other functionalities can also be present in the molecules that are coupled together or other reactions can occur simultaneously in the presence of the cycloaddition reaction. In the brief period since the copper-mediated cycloaddition reaction has been reported, there have been about 50 polymer-based papers that make direct use of the reaction. Many are communications rather than full papers and this is probably a reflection of the urgency to discover the limits of the method's application to polymer science as well as the desire to gain priority for these discoveries. The genesis of this interest can be largely traced back to a meeting of Barry Sharpless and Craig Hawker at a Cornforth Symposium in Sydney. The subsequent collaboration between the respective groups resulted in the dendrimer publication in *Angewandte Chemie*.[11] It and subsequent publications have served to alert the polymer community to the potential of the azide–alkyne cycloaddition. As the cycloaddition is a coupling reaction, essentially all variations have been reported such as end group reaction, side chain or orthogonal reaction, surface coupling, and step growth polymerization. Currently, there are two major themes in the use of azide–alkyne cycloaddition chemistry in polymer science. They are the integration of ATRP and azide–alkyne cycloaddition chemistries and dendrimer synthesis/functionalization.

Polymer Synthesis

The most obvious use of the cycloaddition is with molecules that simultaneously contain both an alkyne and azide group to be clicked together in a head-to-tail step-growth polymerization ['A–Z' (A = Alkyne, Z = aZide) polymerization]. The

simplest molecule in which this might be done is propargyl azide 3 (Caution! N:C 1:1!). Surprisingly, this was reported almost 40 years ago together with 2-propynyl azidoacetate 4 and azidophenyl acetylene 5 without the use of copper(i) (Fig. 5).[46] The monomers spontaneously polymerize at room temperature or with gentle heating to give largely intractable materials which are presumably mixtures of 1,4 and 1,5 triazole regioisomers. Additional chemistry must also occur as indicated by the coloration of products and the liberation of nitrogen in the case of propargyl azide, 3, polymerization.

Once the beneficial properties of copper(i) became known in the cycloaddition reaction it was applied to step-growth polymerizations. In a similar manner to the pre-copper polymerization described above, an acetylene azido terminated polystyrene was itself polymerized in a step-growth polymerization (Fig. 6).[20] This was achieved by the ATRP of styrene with an alkyne-functionalized initiator. The resulting polymer 6 was terminated with bromine. This was displaced with sodium azide (DMF, room temp.) to give an azide–alkyne α-ω heterotelechelic polystyrene 7 which was then step-growth polymerized to give polymer 8 with CuBr in deoxygenated DMF (Fig. 6a). Surprisingly, a significant amount (17%) of starting material remained unconsumed and had probably cyclized rather than polymerized (A–Z polymerization). An improvement in the synthesis was achieved by a reaction of bis-azido α-ω homotelechelic polystyrene 9 with propargyl ether 10 (A–A, Z–Z polymerization) to give polymer 11 with residual monomer/cyclized monomer reduced to only 7% (Fig. 6b). The cyclization hypothesis was strengthened by a later publication where azide–alkyne α-ω telechelic polymer was deliberately cyclized using high-dilution conditions in a solvent-based reaction using CuBr and bipyridine in DMF.[18] The displacement of the terminal halogen from an ATRP prepared polymer is a reaction that is seen in many of the polymer papers that involve the azide–alkyne cycloaddition reaction. This is one of the areas of overlap between ATRP and the cycloaddition reaction and will be discussed later.[13,18,19,21–24,26] The iterative synthesis of triethylene glycol oligomers was achieved by the sequential addition of a triethylene glycol (capped with a propargyl ether and tosylate) to an azido-functionalized triethylene glycol. After reacting together, the tosylate end-group on the resultant oligomer is then displaced by azide and the cycle can be repeated.[47]

An interesting variation of this was the A–A Z–Z step growth polymerization of aryl and fluorenyl dialkynes with diazido-fluorene derivatives to produce a polymer related to the light
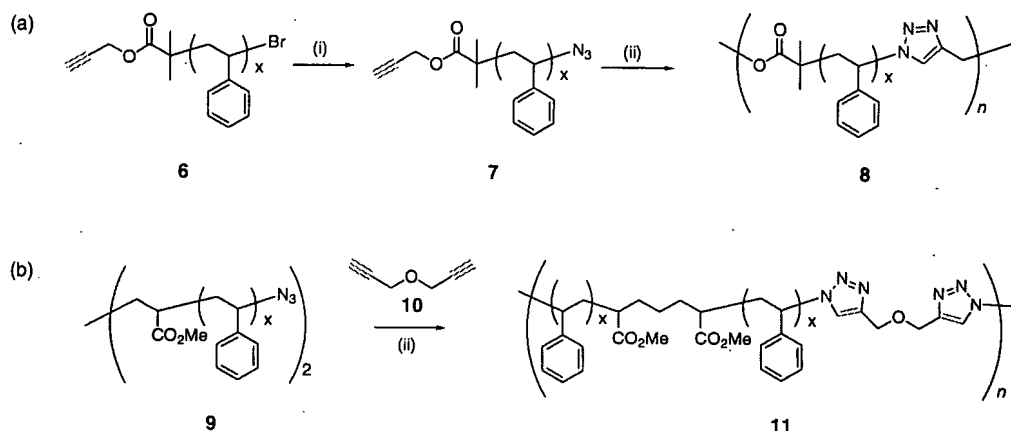
Fig. 6.   The two modes of step growth polymerization of ATRP-derived polymers illustrated by functionalized polystyrene: (a) A–Z polymerization where the azide and alkyne are contained within the same molecule; and (b) A–A Z–Z polymerization where the azides and alkynes are in separate molecules. (i) NaN₃ in DMF, room temperature, 4 h; (ii) CuBr in deoxygenated DMF, room temp.

emitting poly(dialkylfluorene)s.[12] This was a THF/acetonitrile solvent-based cycloaddition using Cu/Cu(OAc)₂ with the ligand 1 at low temperature (−10°C). Unfortunately, the resulting polymers showed absorption spectra that were the superimposition of the monomer units, which indicated no ground state interaction between the components of the polymer. There was poor communication between the fluorene units, thus an extended system of conjugation through the polymer via the triazole units was not formed.

A trehalose–oligoamine polymer was synthesised by A–A Z–Z step-growth polymerization of a diazidotrehalose 12 with a diacetylenic oligoamine 13 (Fig. 7). The idea was to construct a polyfunctional polymer 14. The trehalose provided increased biocompatibility, the triazole unit provided for hydrophobic, van der Waals, and hydrogen bonding with nucleic acids, and the oligo-amine provided for electrostatic DNA binding, phosphate charge neutralization, and cellular uptake.[48] In this instance, one would think that this would be a native application of the cycloaddition reaction because of the water-soluble nature of the polymer components. However, protection and deprotection steps were used in the synthesis so the full simplicity and advantages of the aqueous cycloaddition reaction and the click methodology were compromised.

This step-growth methodology has been applied using polyazides and polyalkynes to give cross-linked materials. Polyvalent monomers were used in an adhesive study for copper and zinc surfaces (Fig. 8).[36] Monomer solutions that contained the azide and alkyne were placed between copper plates which also acted as the source of copper(ɪ) (by comproportionation of Cuɪɪ on the surface) for the cycloaddition reaction. The triazole itself is expected to bind to the copper metal [cf. use of poly(triazole) ligands to stabilize Cuɪ in the cycloaddition reaction[10]], which would further improve adhesion. Thus the entire system is elegantly integrated, and comparable or superior adhesive strengths to commercial glues were reported.[36] The crosslinking of ATRP-derived polymers with tri- and tetra-alkynes for the preparation of degradable model networks has also been reported.[21]

. Azide–alkyne cycloaddition chemistry was found to provide poly(ethylene glycol) (PEG) hydrogel networks using aqueous cycloaddition protocols.[13] PEGs (M_n 3400–10 000) were endcapped with pentynoates which then underwent reaction
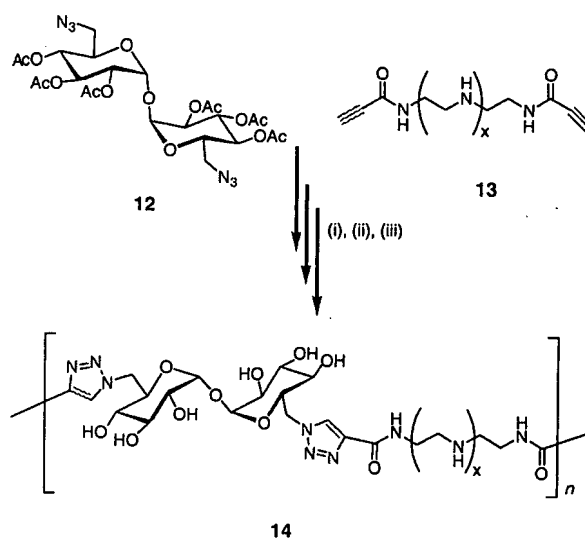

Fig. 7.   Synthesis of trehalose–oligoamine conjugates by azide–alkyne cycloaddition followed by deprotection. (i) CuSO₄/sodium ascorbate, 1:1 BuᵗOH/water; (ii) NaOMe/MeOH; (iii) CF₃COOH/CH₂Cl₂.[48]

with tetraethylene glycol that contained four azide groups to give the hydrogel 15 (Fig. 9). The consumption of the azide and alkynes was very high with only 0.2% of unreacted functional groups detected by fluorescence spectroscopy. The paper also flags a possible concern with copper-based cycloadditions. The aqueous protocol represents an excellent method of coupling water-soluble molecules/polymers together. As the initial reports showed, the chemistry lends itself to biologically active or bio-molecule conjugation. However, polytriazoles coordinate to copper and so the presence of residual copper in triazole-based polymers that are to be used in bio-applications may be a significant issue.[49] In the hydrogel report,[13] copper levels were reduced by extraction with EDTA, however, such a procedure may not be applicable in other cases depending on the other functional groups present. A related example was the formation of degradable model networks through the use of ATRP and azide–alkyne cycloaddition in DMF.[21] As has been commonly
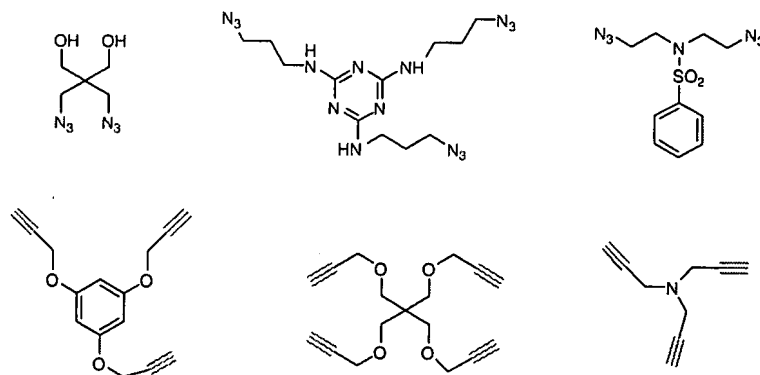
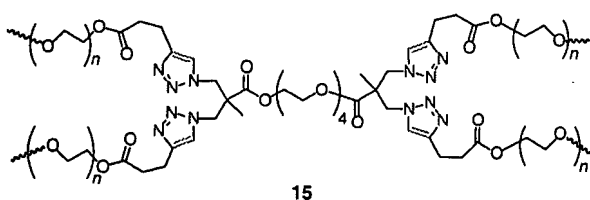Fig. 8. A selection of polyvalent azides and alkynes used as components of copper and zinc adhesives.[36]

15

Fig. 9. Structure of a hydrogel formed by aqueous azide–alkyne cyclo-addition reaction.[13]

Fig. 10. The overlap between ATRP and solvent-based Cu^I-based azide–alkyne cycloaddition.

done, the terminal halogen of the ATRP derived polymer was replaced with azide and the subsequent reaction with a tri- and tetra-alkyne provided the network.[21]

The use of the cycloaddition reaction was investigated to make hyper-branched polymers through the use of dialkyne azides roughly analogous to compound **5** in Fig. 5.[50] As in the case of **5**, the dialkyne azides spontaneously polymerized at room temperature. Despite the nature of the polymer it was found that the ratio of 1,4- to 1,5-triazoles was approx. 60:40. When copper was used to catalyze the reaction in aqueous DMF, a brown insoluble rubber precipitated. It was determined that only 1,4-triazoles had formed and the degree of branching could not be determined. There has been little further examination of cycloaddition in hyper-branched polymers.

End-Group Reactions

Instead of being used for the creation of step-growth poly-mers, the azide and alkyne end groups of polymers can be simply used to end-functionalize polymers or assemble polymer conjugates.[19,23–27,51] Opsteen and Hest coupled blocks of PEG and ATRP-derived polystyrene and poly(methyl methacrylate) together in various combinations to give A–B block copoly-mers as well as an A–B–A triblock.[19] These were performed in organic solvent (DMF) with CuI/DBU. Small excesses of alkyne-terminated polymers were used and unreacted alkynes were either washed out in MeOH (for PEG) or scavenged on an azide-functionalized solid support. The coupling appeared highly efficient with no unreacted azide-functionalized poly-mer remaining. Such a modular approach would appear to make adapting the entire process into a high throughput methodology relatively straightforward, from the synthesis of the polymers, the azide functionalization of the polymers, and to the final cou-pling of the polymers into blocks and their purification. Given the
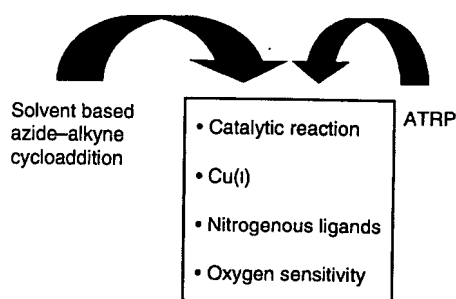
increasing importance of block co-polymers, this area is likely to see further development. One of the few papers that com-bine RAFT[52–54] and azide–alkyne cycloaddition chemistries used the cycloaddition reaction to couple blocks of polymers typically not found together such as polystyrene and poly(vinyl acetate).[42] This was done in THF with CuI and DBU. In a related manner to Opsteen et al., Gao and Matyjaszewski coupled ATRP-derived polymers to tri- and tetra-alkyne core molecules to make three- and four-armed star polymers.[23] They used what is essentially an ATRP catalysis formulation to catalyze the azide–alkyne cycloaddition itself using PMDETA/CuBr with copper metal to overcome an oxygen sensitivity problem. Thus the over-lap between azide–alkyne cycloaddition and ATRP chemistries was complete in this case and highlights the strong relation-ship between them. ATRP and solvent-based click azide–alkyne cycloadditions are catalytic reactions with copper(I) that use nitrogenous ligands and are oxygen sensitive (Fig. 10). A simi-lar integration of ATRP and 'click' azide–alkyne cycloaddition chemistry was reported by Mantovanni et al. using N-alkyl-2-pyridylmethanimine/CuBr in toluene.[27] This relationship between ATRP and the cycloaddition was specifically explored by Golas and Matyjaszewski et al. in a publication where the major ATRP ligands such as PMDETA, Me$_6$TREN, and oth-ers were examined.[55] The following conditions were found to enhance the rate of the cycloaddition; use of a non-coordinating solvent such as toluene (rather than DMF), aliphatic amines like PMDETA (rather than pyridine-based ligands), and tridentate ligands like PMDETA (rather than a tetradentate ligand like Me$_6$TREN). Although ATRP ligands have now been shown to be useful as ligands in azide–alkyne cycloaddition, the use of
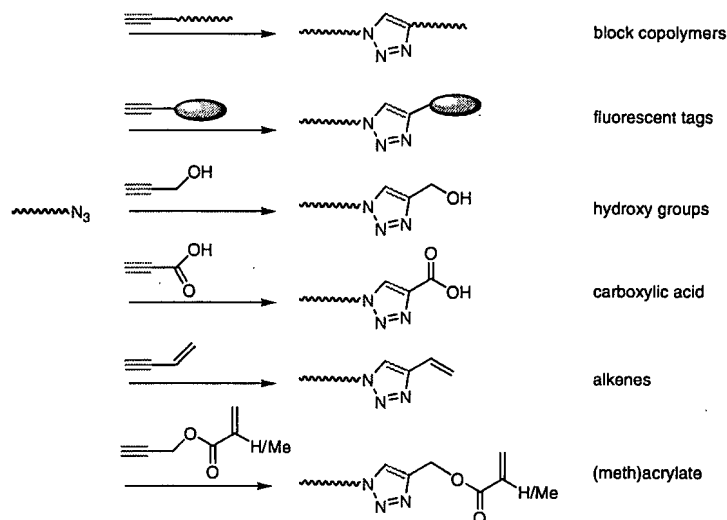
Fig. 11. End functionalization of polymers using azide–alkyne cycloaddition with polymers (to make blocks), fluorophores, hydroxy, carboxy, alkenes, and (meth)acrylate groups.
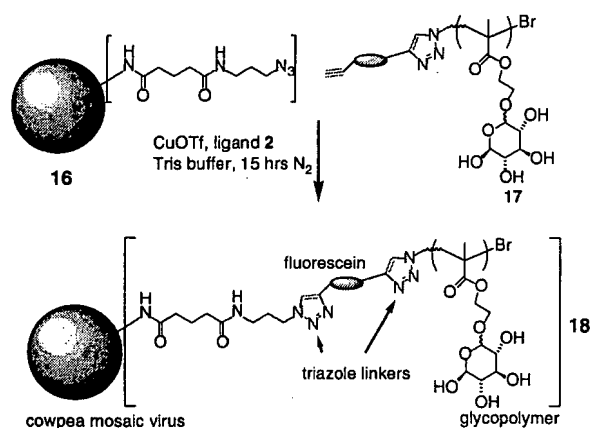

Fig. 12. Functionalization of cowpea mosaic virus with a fluorescently labelled glycopolymer by azide–alkyne cycloaddition.[51]

triazole derived ligands (as used in azide–alkyne cycloadditions) in ATRP has yet to be reported.

Beyond coupling polymers together to make blocks, there several examples of end-functionalizing ATRP derived polymers with a variety of functional molecules and groups. Such molecules have been fluorescent dyes,[27] methacrylate (then used as macromonomers),[25] and functional groups such as hydroxy, acid, and alkenes[26] (Fig. 11). To date the majority of examples use an azide-terminated polymer and couple a functional alkyne to the end rather than the other way around due to the ease of introduction of the azide group to ATRP derived polymers.

A spectacular example of the use of the azide–alkyne cycloaddition reaction in bioconjugation of a virus [cowpea mosaic virus (CPMV)] with a multi-functional polymer has been reported[51] (Fig. 12). This built on the work of Fokin, Sharpless, and Finn et al., where a virus was bioconjugated with a fluorescent dye using the ligand tris(benzyltriazolyl)amine 1 and tris(carboxyethyl)phosphine in place of ascorbate.[5] In the later report, ATRP was used to grow a glycopolymer using an azide

functionalized initiator. This was then coupled (by azide–alkyne cycloaddition) to a fluorescein, which was then coupled (by azide–alkyne cycloaddition) to the virus by a linker. The final coupling of the polymer to the virus used the water-soluble ligand 2, under inert atmosphere with copper(I) triflate and TRIS buffer. Such a structure can also be thought of as a bio-templated star polymer where the multi-valency of the virus provides the core for the attachment of many arms of glyco-polymers. Unlike the earlier report where virus decomposition occurred under standard aqueous cycloaddition conditions using copper and ascorbate, no decomposition was reported with the described method. Thus, the reaction has to be tuned in each individual case.

Side-Chain Functionalization of Polymers

This area has not been as extensively explored as the end functionalization of polymers. Side chain or orthogonal functionalization typically requires the polymerization of an alkyne or azide functionalized monomer. This will result in a polymer with many alkynes or azides pendant from the backbone, which will then undergo a subsequent cycloaddition reaction. It has been found that homopolymerization of monomers that contain terminal alkynes results in crosslinking at medium to high conversion. Sumerlin et al. reported the ATRP of azidopropanol and propargyl methacrylates.[22] They found the homopolymerization of propargyl methacrylate to give multimodal molecular weight distributions and crosslinked networks at moderate to high conversion.[22] A similar result was observed for styrene-derived 4-ethynyl vinylbenzene.[39] This points to the carbon–carbon triple bond participating in the polymerizations. One method to work around this problem is to protect the alkyne in the form of a (trimethylsilyl) alkyne and then convert it into the terminal acetylene with tetrabutylammonium fluoride or basic conditions.[22,39–41] In contrast, 3-azidopropyl methacrylate was polymerized with reasonable control over molecular weight and polydispersity (approx. 1.25–1.5) with the azide groups readily undergoing reaction with a variety of terminal alkynes in deoxygenated DMF or DMSO with Cu(I)Br to give hydroxy, acid, triphenylphosphonium bromide, and 2-bromoisobutyrate side chain functionalities.[22] The terminal alkyne group is less
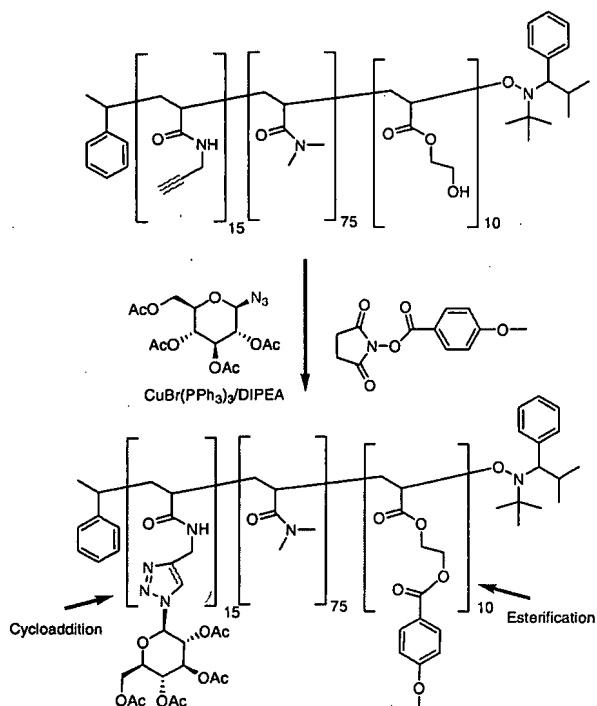
Fig. 13. The side-chain functionalization of a polymer by simultaneous azide-cycloaddition and esterification reactions.[39]

sensitive to non-radical methods of polymerization as demonstrated by the ring-opening co-polymerization of an acetylene-containing lactone with caprolactone by Sn(OTf)₂. The resulting alkyne-functionalized polymer underwent reaction readily with azido-PEGs to give PEG-grafted polyesters.[56] Gheorghe et al. reported the immobilization of propargyl TEMPO on to azide-functionalized polystyrene beads (CuI, THF) to give a reagent for the selective oxidation of aliphatic and benzylic alcohols.[57] Obviously, the TEMPO function cannot be incorporated into a polymer during a radical polymerization; the cycloaddition provided an excellent route to TEMPO-functionalized polymers. Porous beads have been similarly functionalized. Glycidyl methacrylate beads were reacted with azide to provide azide surfaces that allowed coupling to terminal aliphatic or functional alkynes with CuI in acetonitrile, or in water with ligand 1.[58] ATRP and copper-mediated azide–alkyne cycloaddition were combined in a report on the synthesis of glycol polymers.[41] As in the report by Sumerlin and Matyjaszewski et al.,[22] a propargyl side-chained poly(methyl methacrylate) was prepared by ATRP and conversion of a trimethylsilane alkyne. Various azido-sugars were then cycloadded to the polymer with CuBr(PPh₃)₃ and DIPEA. Malkoch and Hawker et al. demonstrated functionalization of a polymer that contained both alkyne and hydroxy groups by azide–alkyne cycloaddition (CuBr(PPh₃)₃, DIPEA, THF) and esterification chemistries occurring simultaneously (Fig. 13) as an example that further underlines the selectivity of the cycloaddition reaction in the presence of other reactive functionalities and reactions.[39]

Dendrimer and Supramolecular Application of Azide–Alkyne Cycloaddition Chemistry

The use of copper-mediated azide–alkyne cycloaddition in dendrimers mirrors that for conventional polymers. The reaction

has been used for the synthesis of dendrons and dendrimer skeletons,[11,37,59] for surface or end-functionalizing the periphery of the dendrimer,[16,35,60] and coupling of dendrimers in various ways.[37,38,61–64] The initial report of triazole-based dendrimers by the groups of Hawker, Fréchet, Voit, and Sharpless used the convergent methodology[65] of dendrimer synthesis using the reaction of two azides with a di-alkyne alkyl chloride. The resultant bis-triazole alkyl chloride was converted into an azide, and so the dendrons were built up with final coupling to a tri-alkyne core to give the dendrimer.[11] Standard aqueous reaction conditions with copper sulfate and ascorbate were used for lower generation dendrons, while organic-solvent-based microwave conditions were needed for higher generations depending on the periphery end groups. The use of excess dendrons and chromatographic purification in the synthesis of the final dendrimers were not needed. Logically, it is of interest if dendritic triazoles[11] can act as efficient ligands in the azide–alkyne cycloaddition reaction itself, although the water insolubility of dendrimers may be a problem. Dendrimers have also been made divergently using copper sulfate and ascorbate in a variety of solvents (water, ethanol, and DMSO).[37] From there, the method has been extended not only to coupling convergently synthesized dendrimers to linear polymers,[40] but also to divergent dendrimers on a polystyrene backbone to give doubly dendronized linear polymers.[38]

The functionalization of the outer layer of dendrimers has received some detailed attention. Similar to the end-functionalization of linear polymers described earlier, dendrimers have been chain-end functionalized with a diverse range of functional groups such as azidothymidine (AZT),[16] sugars,[16,59–61] peptides,[35] non-linear dyes,[16] dendrons,[16,38] and fluorophores.[16,61] Unlike the initial report on azide–alkyne cycloaddition dendrimer synthesis,[11] chain-end functionalization of dendrimers has appeared to frequently require an excess of the functional azides and purification by chromatography[16,59,60] with yields ranging from poor to excellent. There appears to be a preference to have the alkyne on the dendrimer and the azide on the functional molecule, but there is one report of the azide on the periphery of the dendrimer and the alkyne on the carbohydrate (Fig. 14).[59] The functionalization of dendrimers with sugars is a particular focus due to the synergistic fit of click chemistry with sugar chemistry as the reaction can be water based with no protecting groups on the sugar required. The general use of azide–alkyne cycloaddition to decorate substrates (not just dendrimers) with sugars is likely to be a significant area of ongoing research. What is particularly notable in the dendrimer field is the mixing of the divergent and convergent methods of dendrimer synthesis in many of the papers using the azide–alkyne cycloaddition reaction. There are examples of convergently made dendrimers undergoing a reaction on the periphery in the last step[16,35,60] and divergently made dendrons being coupled together at the focus as a last reaction.[63,64,66,67] All this is made possible by the selectivity inherent in the azide–alkyne cycloaddition reaction. From dendrimers, the cycloaddition reaction has been used in supramolecular assemblies to functionalize the core or shell of micelles of block co-polymers.[68] The availability of the two regions was shown through the use of alkyne-functionalized fluorophores. The technique was then extended to cross-linking organic nanoparticles themselves.[69] Iron oxide nanoparticles functionalized with azide groups have successfully undergone reaction with alkyne end-functionalized polymers.[70]
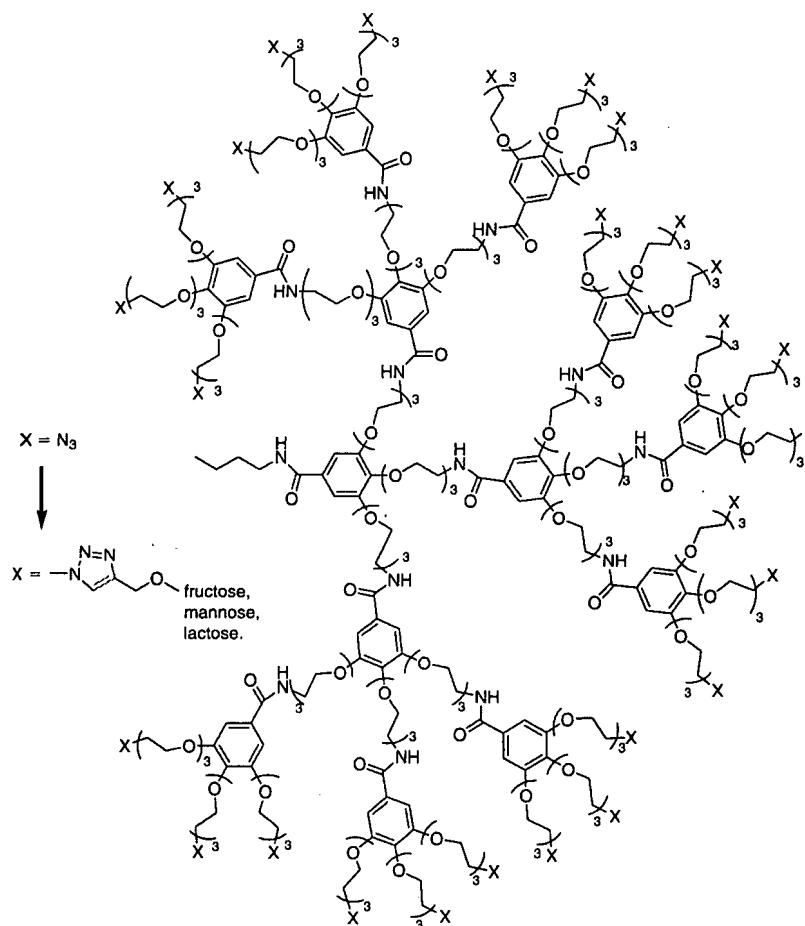
Fig. 14. The chain-end functionalization of a convergently synthesized dendrimer with sugars using copper-mediated azide–alkyne cycloaddition reactions.[59] Reaction conditions; 2 molar excess of alkyne functionalized sugar, $CuSO_4 \cdot 5H_2O$, sodium ascorbate Bu^{t}OH/water, room temperature, 24–72 h.

Surface Azide–Alkyne Cycloadditions

There are only a handful of reports concerning the use of the azide–alkyne cycloaddition reaction for coupling to or modifying a surface,[49,71–77] and of these only two involve non-biological polymers.[76,77] However, this area is rapidly expanding due to the broad general need for mild and efficient surface immobilization strategies for microarray and other biological applications. Initial reports by Collman et al. and Lummerstorfer and Hoffman, were the functionalization of a gold[71] or silica[72] surfaces with an azide group by an alkyl chain and appropriate anchoring chemistry. Acetylenic ferrocene (CuSO$_4$·5H$_2$O, ascorbate, water, room temperature) and simple alkynes (no catalyst, ethanol, 20 or 70°C) were reacted with the surface azide groups. The strong distinctive azide stretching mode in the IR at ~2100 cm^{-1} was used to monitor the progress of the reaction. Both reports describe that the reaction occurs under mild conditions *without copper catalyst* if the acetylene is electron poor and the azide electron rich. Although copper-free cycloaddition provides little regioselectivity over the triazole isomer formed, steric constraints on the surface side of the azide may favour 1,4-substitution. This possible sterically induced regioselectivity needs to be experimentally validated in the future.[72] The gold monolayer approach was further extended to the immobilization of oligonucleotides[73] and carbohydrates.[78] Oligonucleotides end-functionalized with a terminal acetylene were coupled to the surface bound azides using Cu^I in aqueous DMSO with the ligand 1 and ascorbate. Copper(i) in the presence of oxygen has previously been shown to react with DNA (cf. reference 9 within the report) and it was feared that it would do the same in this case. Thus, the ligand 1 was used to not only speed the reaction but also suppress copper(i)-generated reactive oxygen species. Although a control reaction was not reported to confirm DNA damage under ligand free cycloaddition conditions, rapid ligand 'protected' immobilizations (30 min) were obtained. It was also found that the surface azide content had to be diluted as the original high density of surface probe oligonucleotides reduced subsequent hybridization with target oligomers in solution (~24%) through steric hindrance. Once the surface azide content was reduced, a very high hybridization efficiency was obtained (~96%). Now that proof of principle has been obtained it is of interest if the method can be integrated into micro-array spotting protocols.

The emphasis on biomolecule immobilization continued in reports on the immobilization of peptides and carbohydrates.[74,75] Lin et al. reported the immobilization of green fluorescent protein (among others).[75] Importantly, they found
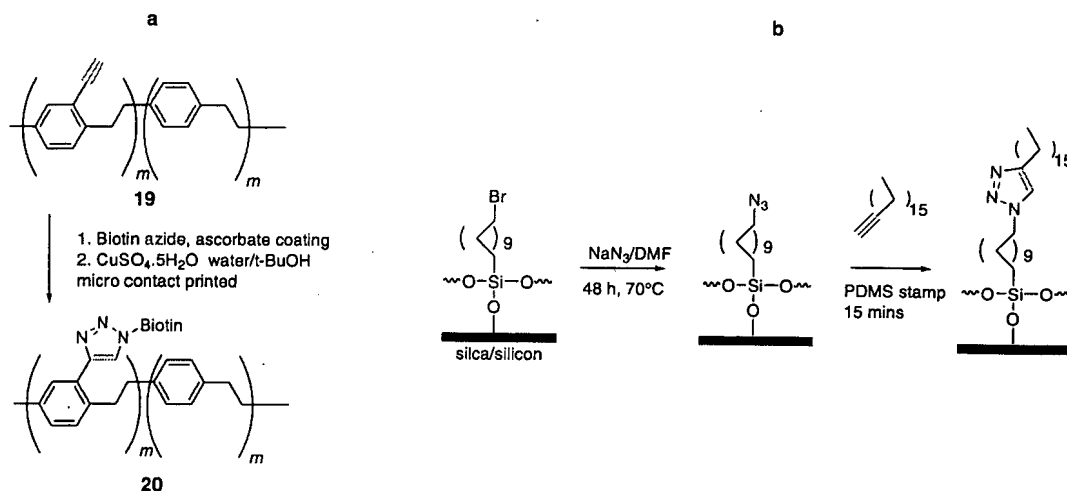
Fig. 15. (a) Immobilization of biotin azide to a surface bound alkyne using contact microprinting and copper-mediated azide–alkyne cycloaddition;[74] and (b) rapid copper-free cycloaddition microprinting with surface-bound azide and alkyne-loaded PDMS stamp.[49]

in such systems that it was much better to have an azide-functionalized surface and alkyne-functionalized biomolecule rather than the other way around. This appears to be a result of the lower availability of the alkyne to form the copper acetylide when tethered to a surface. It is of interest to see if this surface effect could be reduced by increasing availability and mobility of a surface-bound alkyne through the use of a long tether between the surface and alkyne. Certainly surface bound alkynes can undergo cycloaddition reactions; Sun et al. reported elaborately engineered surface-bound alkynes to immobilize carbohydrate and proteins.[74] They used both a triazole-based ligand (similar to 1) to speed the reaction and prevent oxidative coupling, and copper(0) in place of ascorbate to prevent cleavage of disulfide bonds. Nandivivada et al. also created surface bound alkynes by chemical vapour deposition polymerization of 4-ethynyl[2,2]paracyclophane.[76] The resulting alkyne functional polymer 19 was micro-contact printed in an interesting protocol by coating the polymer with ascorbic acid and a biotin azide followed by a PDMS stamp inked with copper sulfate solution (room temperature, 12–18 h, Fig. 15a). Rhodamine-labelled streptavidin verified the success of the click-immobilized biotin to the polymer 20. This has been followed up by another report of micro-contact printing of alkynes onto an azide-functionalized surface but it was found that no copper was needed even for alkynes that were not electron deficient (Fig. 15b).[49] The speed of reaction was thought to be due to the high local concentrations of reagents and the result is one that will attract a lot of attention and follow-up research.

An example of purely organic polymer based surface click chemistry is by Such and Caruso et al.[77] They created ultra-thin polymer multi-layers by laying down sequential layers of poly(acrylic acid) polymers that contained a small amount of either alkyne or azide-functionalized acrylate (~14% and ~16%, respectively). Each layer was covalently joined by triazole formation before an additional layer was laid down, thus stabilizing the layer-by-layer structure. This reaction can be considered a special case of side-chain functionalization of a polymer. Here the polymer is being essentially functionalized by another polymer. Interestingly, copper was bound into the layers, presumably because of complexation to the polytriazoles,
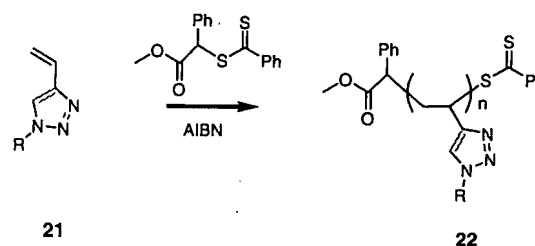

Fig. 16. New polymerizable triazoles with tuneable properties.[79]

and this allowed monitoring of the formation of the layers by UV-vis spectroscopy. In general, the presence of residual copper, of course, may be a double-edged sword if biological applications of the technique are sensitive to copper. The use of a good water-soluble ligand in such reactions would be expected to help minimize copper retention in addition to speeding the reaction. Alternatively, post-cycloaddition removal of copper with an efficient copper scavenger like EDTA might be prove useful as it did in the microgel report.[13]

Triazoles as Monomers

Although there has been much work using the copper-mediated azide–alkyne cycloaddition for the functionalization of polymers, there has been little consideration of the use of a triazole as monomer itself. There is currently a single report of a polymerizable triazole 21;[79] it is also one of the few reports[42,77,79] that make use of RAFT to control the polymerization with azide-alkyne cycloaddition chemistry. The compounds combine the aromaticity of styrenic monomers and the functionality of acrylates (Fig. 16). For example, a triazole group can provide stiffness and polarity to give increased glass transition temperature and solubility in protic solvents. The nature of the substituent provides further scope of tuning T_g and solubility. For example, in Fig. 15, when R is phenyl, the polymer 22 has a T_g of 148°C and solubility in ethanol. It will be of interest to see how the properties of dendritic and chain-growth polytriazoles compare in future work.

Conclusions and Possibilities

In the relatively brief time since the initial reporting of the copper-mediated form of the azide–alkyne cycloaddition, it has found a home in the tool box of the polymer scientist as evidenced by many imaginative reports described above. It has been shown capable of generating polymers and functionalizing them in a variety of ways. The organic solvent-based method has found a natural partner in ATRP with which it shares a metal, ligands, solvents, and oxygen sensitivity. Similarly, the aqueous-based method has found a natural polymer application in creating glyco-polymers where unprotected sugars can be coupled to polymers largely without fear of side reactions. The use of the cycloaddition with, or to make, water-soluble polymers will continue because of potential drug-delivery applications. Interest in the synthesis of polymer–biomolecule conjugates would be expected to expand for the same reason. The fidelity of the method to make triazole dendrimers is also quite remarkable. The high selectivity of the reaction is such that other reactions can proceed simultaneously with it. The participation of the azide–alkyne cycloaddition in orthogonal approaches to polymer functionalization has been illustrated and makes the discovery of other equally selective click reactions of great interest. The incorporation of azide–alkyne cycloaddition with the RAFT living radical polymerization technique is another relatively unexplored area that one would expect to see increasing numbers of reports on before too long.

As with any new synthetic approach, the limits of its applicability are being probed. The simplicity of the method, particularly the aqueous copper sulfate/ascorbate method, does not always translate to polymer applications. Although water insolubility of reactants appears not to be a problem in small molecule synthesis reactions, many polymers appear to need organic solvent conditions, which are more awkward to work with. The utility of the reaction would be greatly expanded if an aqueous-based protocol could be found that worked with typical styrenic and acrylate-based polymers. The biphasic CH_2Cl_2/water procedure described earlier is one possibility that could be assessed for polymer applicability. Another desirable discovery would be a water-soluble ligand without oxygen sensitivity; this would allow the acceleration of reactions but minimize contamination of polymers with ligands. In addition, such ligands may be of benefit to help prevent copper from being bound into triazole rich polymers (or otherwise causing problems) be they on surfaces, neat, or conjugated to large biological molecules or entities like viruses. Although the cycloaddition reaction can be extremely facile, the effort in creating the azide and alkyne functionalities on the polymers and functional molecules in order to do the cycloaddition has to be considered. A particular case in point is the reported reactivity of terminal alkynes to radical reactions. This has necessitated the use of trimethylsilyl alkynes in the preparation of polymers followed by conversion into the acetylene in order to provide the alkyne-functionalized polymers ready for subsequent cycloaddition chemistry.

It is perhaps a little ironic that the discovery of the catalytic properties of copper(i) has ignited re-examination of the copper-free azide–alkyne cycloaddition. This has resulted in some significant discoveries in performing the reaction that eliminate the need to use copper in some surface reactions. Copper-free protocols are of great potential value in biological applications as it simplifies the procedure and eliminates the potential adverse effects because of copper or ascorbate. The rapid, copper-free reaction in surface microcontact printing would appear suitable to find widespread adoption in micro-array and general surface modification applications.

If the only use of the azide cycloaddition is as a coupling methodology, then its continued use is already ensured. However, the ability of the 1,2,3-triazole moiety to provide additional functionality such as hydrogen bonding, coordination, or bioactivity, etc., has barely been examined in a polymer context. Discovery of such additional functionality would serve to further increase the impact of the azide–alkyne cycloaddition reaction and may represent the next phase of research efforts and reports.

Acknowledgments

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Azide-Modified Graphitic Surfaces for Covalent Attachment of Alkyne-Terminated Molecules by "Click" Chemistry

Anando Devadoss and Christopher E. D. Chidsey*

Department of Chemistry, Stanford University, Stanford, California 94305

Received February 22, 2007; E-mail: chidsey@stanford.edu

Functionalization of graphitic surfaces is of fundamental and technological importance because carbon-based materials are used in the fields of energy conversion,[1] molecular electronics,[2] and sensors.[3] A method to graft aryl molecules onto graphitic surfaces by electrochemical reduction of the corresponding aryl diazonium ions is widely used.[4] Aryl radicals formed react with the graphene sheets to form direct C—C bonds.[5] However, the radical intermediate leads to formation of multilayer structures of aryl molecules ranging from 0.7 to 15 nm in height on the surface.[6] Such multilayers are not ideal for coupling redox molecules to the electrode because of the ill-defined electron-transfer path. Methods recently developed to couple alkynes[7] require high-temperature treatment of up to 400 °C, and alkenes[3] require photochemical treatment, limiting these methods to molecules stable to high temperature and radiation, respectively. Other general methods of coupling molecules to graphitic surfaces are oxidative in nature.[5,8] Oxidative methods form various oxygenated functional groups on graphite,[9] making it difficult to understand the exact nature of the linker between the surface and the molecule.

We explored the possibility of preparing azide-modified graphitic surfaces to couple molecules in a highly selective orientation and with a well-defined linker. Azides allow coupling of alkyne-terminated molecules via Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition,[10,11] popularly known as a "click" reaction. This reaction is extremely selective and high yielding while utilizing very mild reaction conditions. The product of the reaction is the stable heterocyclic linker, 1,4-disubstituted 1,2,3-triazole. Azides and alkynes are essentially inert to molecular oxygen, various solvents including water, and common reaction conditions in organic synthesis. These advantages are particularly useful for attaching multifunctional molecules to surfaces[12] and suggest that an azide-modified graphitic surface would be a highly versatile platform for attachment of a variety of functional molecules.

In this communication, a one-step chemical method to prepare azide-modified graphitic surfaces is reported using iodine azide as the reagent.[13,14] The azide groups are introduced at the graphitic edges, presumably via a Hassner-type addition of IN_3 to the double bonds and subsequent elimination of HI (Figure 1). X-ray photoelectron spectroscopy (XPS) and cyclic voltammetry studies were used to characterize the azide-modified graphitic surface.

Pyrolyzed photoresist films formed on silicon substrates were used as graphitic surfaces.[15] The surfaces were modified with azides by immersing in an acetonitrile solution containing 10 mM iodine azide. An XPS survey scan of the iodine azide-treated graphitic surface showed nitrogen peaks corresponding to the presence of azides. Peaks due to iodine atoms corresponding to only 10% of the surface coverage of azides (vide infra) were also observed. These observations suggest that addition of iodine azide to the double bonds at the graphene sheets is followed by a spontaneous elimination of hydrogen iodide to form aromatic azides. The surface coverage of the azide groups was determined to be $(4.4 \pm 1.3) \times$
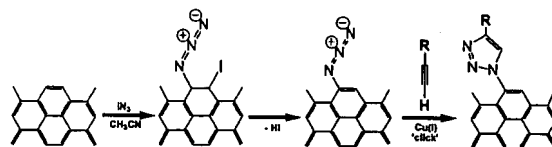

Figure 1. Scheme of the modification of graphene sheets with azides and coupling to terminal alkynes.
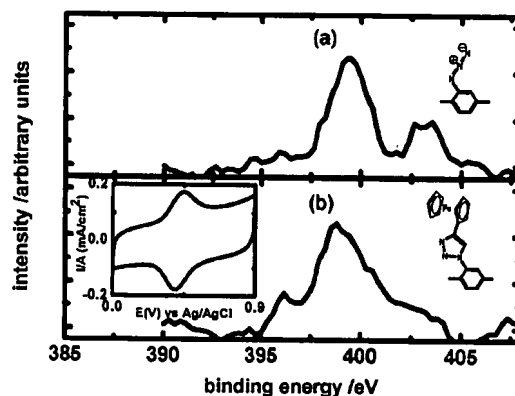

Figure 2. N1s XPS data obtained at a graphitic surface (a) before and (b) after click reaction with ethynylferrocene. The inset shows a cyclic voltammogram obtained at 5 V/s after attachment of ethynylferrocene.

10^{13} molecules/cm² for an immersion time of 20 min. Extending the immersion time to 60 min gave an azide surface coverage of $(7.3 \pm 1.4) \times 10^{13}$ molecules/cm². Further increase up to 16 h did not show any significant increase in the surface coverage. During the course of iodine azide treatment and subsequent reactions, the surface coverage of oxygen remained at the value obtained after pyrolysis of the photoresist, around 2.2×10^{14} atoms/cm².

Figure 2a shows the XPS N1s data obtained at an iodine azide-treated surface. There are peaks at 399 and 403 eV with a peak area ratio of 2:1 as expected for azide groups on the surface.[16] Figure 2b shows the XPS data obtained at the same surface after undergoing the click reaction with 20 μM ethynylferrocene in 3:1 DMSO/H₂O solution containing 400 μM Cu(I)(tris(benzyltriazolyl-methyl)amine)(NO₃) as catalyst. The absence of the peak at 403 eV and broadening of the peak at 399 eV is consistent with formation of a 1,2,3-triazole. The area under the broader peak at 399 eV after the click reaction is equal to the area under the peaks at 403 and 399 eV before the click reaction. These observations suggest that the azide present on the surface forms the 1,2,3-triazole with 100% yield. The presence of ferrocene on the surface was confirmed by the XPS $2p_{3/2}$ and $2p_{1/2}$ peaks for iron at 706 and 720 eV, respectively (see Supporting Information). The azide was stable for several minutes of exposure to the Al Kα radiation. After 10 min, the ratio of the area of the 399 eV peak to the 403 eV
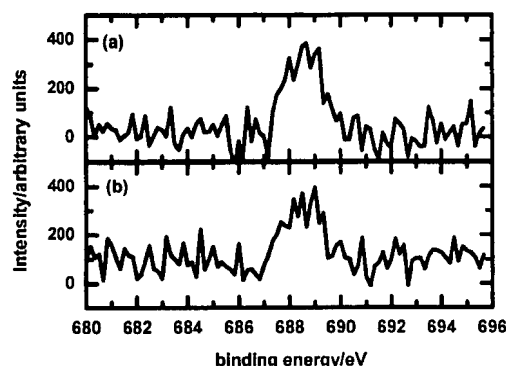
Figure 3. F1s XPS data (a) before and (b) after treating a 1-ethynyl-4-(trifluoromethyl)benzene-modified surface with 1 M HCl at 55 °C for 60 min.

peak decreased from 1:2 to 1:3. We presume that extensive exposure to X-radiation causes elimination of dinitrogen.

The inset of Figure 2b shows the cyclic voltammetric data obtained at a ferrocene-modified graphitic surface in 1 M aqueous perchloric acid. A well-defined redox peak ($E° = 0.42$ V vs Ag/AgCl/KCl) was observed. The peak current was found to vary linearly with scan rate from 1 to 10 V/s and is consistent with a surface-immobilized ferrocene. The full-width at half-maximum of the anodic and cathodic peaks were found to be roughly 0.15 V, approximately 50% greater than the ideal Nernstian width,[17] suggesting some heterogeneity or interaction among the ferrocenes. The charge under the reduction and oxidation peaks gave a surface coverage of 2.0×10^{13} molecules/cm². This surface coverage value agrees with the values obtained for azides using XPS analysis. No peaks corresponding to the ferrocene redox couple were observed when an azide-modified graphitic surface was treated with ethynylferrocene in the absence of the Cu(I) catalyst or when a bare graphitic surface was immersed in a solution containing ethynylferrocene and the Cu(I) catalyst. The voltammogram of the azide-modified surfaces was similar to bare surfaces, suggesting the residual iodine is not electrochemically oxidized. Edge-plane graphite has also been treated with iodine azide and subsequently coupled to ethynylferrocene by the click reaction. Higher coverages of up to 1.4×10^{14} molecules/cm² were obtained. Similar experiments on basal-plane graphite did not show a surface-immobilized ferrocene redox couple. These results support our model of azides attached at the edges of graphene sheets, as shown in Figure 1.

The azide-modified graphitic surfaces were treated with a solution of 20 μM 1-ethynyl-4-(trifluoromethyl)benzene in 3:1 DMSO/H$_2$O containing 400 μM Cu(I)(tris(benzyltriazolylmethyl)amine)(NO$_3$) as a catalyst to form a (trifluoromethyl)benzene-modified surface. Figure 3a shows the F1s XPS peak due to fluorine atoms present on this surface. A surface coverage of 1.5×10^{13} molecules/cm²

was estimated for the (trifluoromethyl)benzene groups using the XPS peaks of fluorine and carbon (see Supporting Information). Figure 3b shows the fluorine peaks observed at the same surface after an acid hydrolysis treatment (1 M HCl for 60 min at 55 °C). No significant change in fluorine or oxygen surface coverage was observed, suggesting the triazole linker is stable to acid hydrolysis.

In conclusion, a method to couple functional molecules to graphitic carbon surfaces through click chemistry has been developed. The wide variety of functional groups that are orthogonal to the click reaction and the stability of the 1,2,3-triazole promise a wide range of applications for these surfaces.

Supporting Information Available: Experimental procedures for preparing graphitic surfaces, azide-modified graphitic surfaces, and ethynylferrocene-modified and 1-ethynyl-4-(trifluoromethyl)benzene-modified graphitic surfaces. XPS data showing peaks due to iron on ferrocene-modified surface. XPS data showing the fluorine region before and after modifying the surface with (trifluoromethyl)benzene. XPS data showing the peaks due to C, N, O, and F before and after 1 M HCl hydrolysis. Surface coverage calculations for azide, I, O, and (trifluoromethyl)benzene groups. This material is available free of charge via the Internet at http://pubs.acs.org.

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A Simple Procedure for Connecting Two Carbohydrate Moieties by Click Chemistry Techniques

Sébastien G. Gouin,[a] Laurent Bultel,[a] Céline Falentin,[a] and José Kovensky*[a]

We describe a procedure to link two saccharides by click chemistry techniques. This methodology allows the generation of new molecules in which two carbohydrates are connected through a triazole ring.

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Introduction

Carbohydrate–protein interactions have been extensively investigated. Oligosaccharides serve to mediate a host of biological events, including cell–cell recognition, adhesion, and modulation of signal transduction pathways.[1] Many proteins and lipids are glycosylated and this affects the functions of the proteins to which they are attached.

In the area of multivalency, the development of multivalent carbohydrates of varying size as effectors of biological processes through clustering of receptors has been a topic of interest. Generally, carbohydrates involved in the binding are connected together through linkers.[2]

The Huisgen 1,3-dipolar cycloaddition between azides and alkynes to afford triazoles is probably the most powerful "click" reaction for such linkages.[3] Interest in this reaction became clear after the recent discovery of the advantages of Cu^I as catalyst, reported independently by Sharpless's[4] and Meldal's[5] groups. This reaction in part owes its usefulness to its high compatibility with functional groups (alcohols, carboxylic acids, amines) in different solvent systems, including water. In the field of carbohydrate chemistry, click chemistry has been used for the synthesis of glycoconjugates[6,7] and carbohydrate macrocycles[8] in which a sugar possessing an azido function is grafted onto a saccharide,[9] a peptide,[10] or a polymeric chain.[11] This methodology has also been employed for the synthesis of glycosidase inhibitors.[12]

We are currently involved in a research program dealing with the construction of new probes for studying multivalency in carbohydrate–protein interactions. In this context, it was necessary to develop a general method to connect carbohydrates bearing a variety of protecting groups. Recent success in the synthesis of glycoconjugates[10,13] by "click chemistry" inspired us to envisage this approach to constructing our molecules, and here we describe a fast procedure to connect saccharides through triazole rings (Scheme 1).
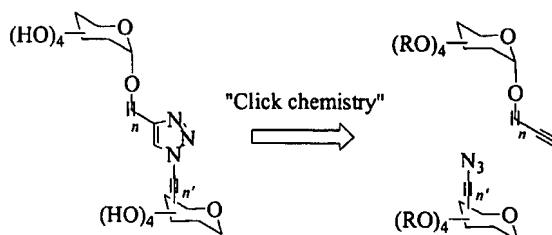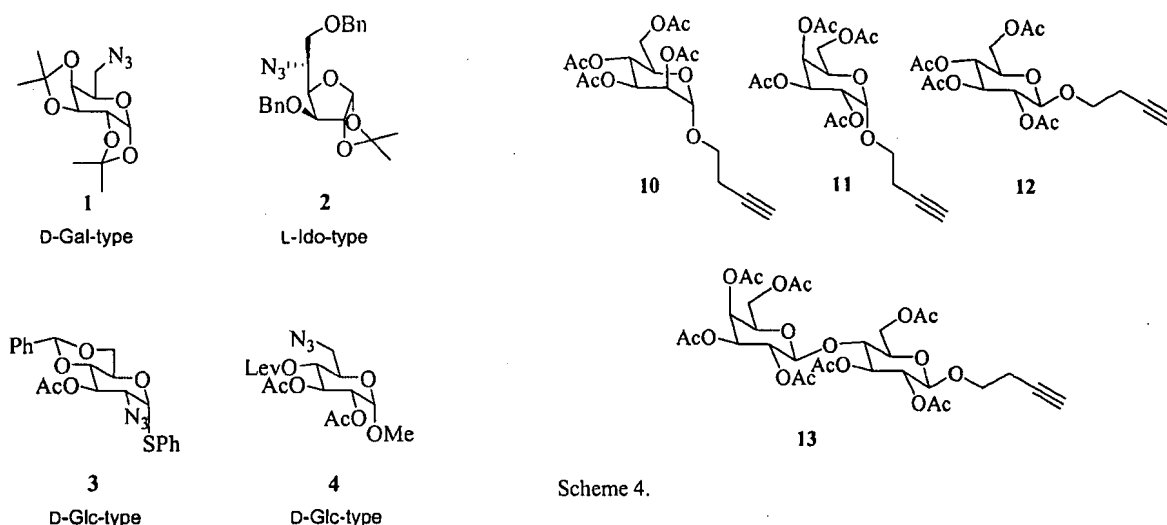

Scheme 1.

Results and Discussion

To demonstrate the broad compatibility of our technique, we have synthesized four azidoalkyl-saccharides (Scheme 2). Azides have been introduced at different positions (C-2, C-5, C-6) in carbohydrate derivatives in their pyranose or furanose forms, in the presence of a range of protecting groups commonly used in oligosaccharide synthesis.

Compounds 1 and 2 were obtained as described previously,[14,15] while the azido derivative 3 was prepared by standard acetylation (pyridine/acetic anhydride, 96% yield) of the 3-OH precursor, easily obtained in five steps from glucosamine, as previously reported.[16]

Azide 4 was synthesized as shown in Scheme 3. Regioselective reductive opening of the 4,6-benzylidene acetal 5[17,18] with TFA/TES gave the known alcohol 6,[19] which was converted into 7 by treatment with an excess of levulinic anhydride.[20] First attempts to remove the benzyl group were conducted with Pd–C in methanol under positive hydrogen pressure. Unfortunately, though, compound 8 was

[a] Laboratoire des Glucides UMR 6219, Université de Picardie Jules Verne,
33 rue Saint-Leu, 80039 Amiens, France
Fax: +33-3-22-82-75-68
E-mail: jose.kovensky@u-picardie.fr

Scheme 2.

Scheme 4.

unstable under these conditions and migration of the Lev group from O-5 to O-6 occurred. The same approach in THF, however, allowed us to isolate **8** in quantitative yield. After conversion of the hydroxy group into the iodide **9** by treatment with PPh$_3$/I$_2$/Im,[21] the synthesis of **4** was achieved by addition of LiN$_3$ in DMF.

Conversely, four different alkynyl saccharides were selected: the mannoside **10**,[22] the galactoside **11**, the glucoside **12**,[23] and the lactoside **13** (Scheme 4), all easily obtained from the corresponding peracetylated sugars by treatment with but-3-yn-1-ol in the presence of boron trifluoride etherate.[23]

The first attempted cycloadditions between saccharides **4** and **10** are summarized in Table 1 and Scheme 5. Nitrogen donors including bases such as DIPEA and some solvents such as acetonitrile have been reported to improve results in Cu^I-catalyzed alkyne–azide coupling by helping to prevent degradation of Cu^I by disproportionation or oxidation.[24] In our hands, these experimental conditions were inappropriate, no reaction having occurred after 2 h in THF with a catalytic amount of Cu^I (Entry 1). In the presence of an excess of CuI (Entry 3), however, the expected compound **14** was obtained in moderate yield after 4 h in THF (En-

try 2) or 1 h in acetonitrile, although the reactions did not go to completion. To overcome the instability of Cu^I, it was generated by in situ reduction of Cu^{II} salts (copper sulfate) with the aid of sodium ascorbate,[25] but no reaction had been observed in sec-butanol or in anhydrous DMF after 3 and 2 h, respectively (Entries 4 and 6). Fortunately, though, we observed dramatic increases in the reaction rates when water was added to the sec-butanol or DMF (Entries 5 and 7), and only the expected triazole **14** was formed (71% and 88% yields, respectively, after flash chromatography). These results demonstrate the high sensitivity of the reaction to the experimental conditions and corroborate precedent reports on the positive effect of water.[26]

As previously observed, the catalyzed Huisgen reaction was found to be highly regioselective, yielding 1,4-disubstituted 1,2,3-triazole-containing carbohydrates.[5] In each cycloadduct, the olefinic proton associated with the 1,2,3-triazole moiety was identified as a singlet between δ = 7.25 and 7.65 ppm, while allylic methylene resonances were observed around δ = 3 ppm. ^{13}C NMR analysis of the compounds were also carried out: the large Δ (δ_{C4}–δ_{C5}) values for the different triazoles, ranging from 20.4 to 22.5 ppm, corroborated the 1,4-disubstituted structure, since much smaller values would be expected for 1,5-disubstituted regioisomers.[27]
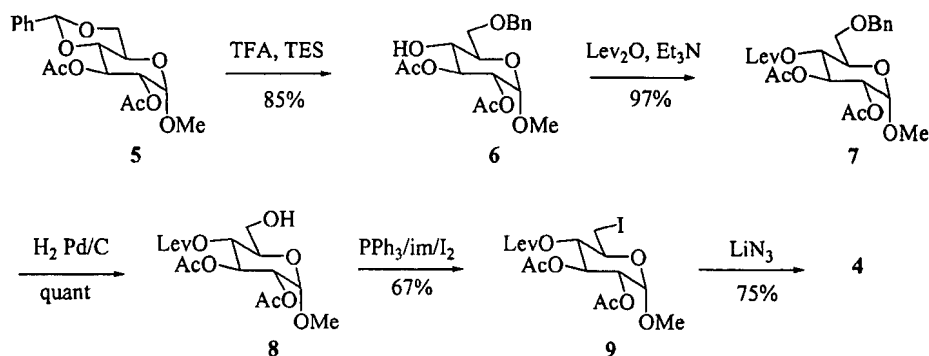
Scheme 3.

Table 1. Evaluation of different conditions for the cycloaddition.

Entry	Equiv. of alkyne	Equiv. of DIPEA	Na ascorbate	Catalyst	Solvent	Time / h	% Yield
1	1	3	–	0.2 equiv. CuI	THF	2	no reaction
2	1.2	4	–	0.2 equiv. CuI	THF	4	55
3	1	3	–	2.0 equiv. CuI	CH$_3$CN	1	49
4	1	–	0.4 equiv.	0.2 equiv. CuSO$_4$	sBu	3	no reaction
5	1	–	0.4 equiv.	0.2 equiv. CuSO$_4$	sBu/H$_2$O	1.1	71
6	1	–	0.4 equiv.	0.2 equiv. CuSO$_4$	DMF	2	no reaction
7	1	–	0.4 equiv.	0.2 equiv. CuSO$_4$	DMF/H$_2$O	1.5	88
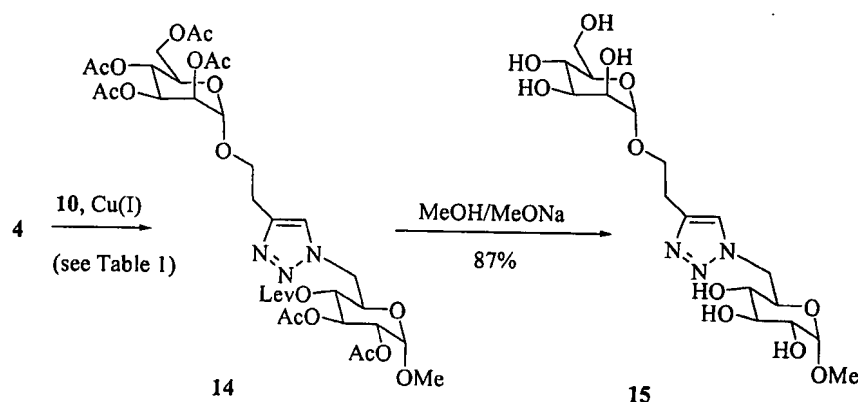
Scheme 5.

Removal of the protecting groups from **14** with a catalytic quantity of sodium methoxide in methanol gave the compound **15** in 87% yield. The triazole ring was not affected by the basic conditions and no degradation occurred.

The best experimental conditions (Table 1, Entry 7) found were applied to couplings of azido sugars **1–4** with alkynyl derivatives **10–13**, and the results are summarized in Table 2.

All the reactions had gone to completion within 5 h at room temperature, with use of equimolar ratios of azide and alkyne and catalytic quantities of copper sulfate. Yields ranged from 70 to 100% after flash chromatography (Table 2). Different cycloaddition trials on compound **4** showed similar yields independently of the α,β stereochemistry of the alkynyl saccharide employed (Entries 1, 2, 3). The reaction time with alkynyl lactoside **13** was slightly higher because of its lower solubility in the DMF/water mixture (Entry 4). Notably, compound **4** (Entry 6) contains a thiophenyl group, which can be activated for further glycosylation. Under our conditions, the intermolecular 1,3-dipolar cycloadditions tolerated the presence of a variety of protecting groups (i.e., acetate or levulinate esters, benzyl ethers, benzylidene or isopropylidene acetals). Azide groups linked to secondary carbons atoms, on the other hand, provided the corresponding oligosaccharide analogues in very good to excellent yields (Entries 6 and 7).

In order to modulate the distance between the monosaccharides, we connected the azido group through a spacer (Scheme 6). Alkylation of **8** with the azide derivative **22**, freshly prepared as described previously,[28] was problematic

in the presence of base, probably due to the sensitivity of acetates to the alkaline medium. The reaction was therefore conducted in the absence of base and under vacuum, by a method previously described.[29,30] Compound **23** was isolated in 55% yield. In spite of the moderate yield obtained, this reaction is particularly interesting as the starting material can be recycled. The general procedure for the click reaction was employed and product **24** was isolated in 72% yield.
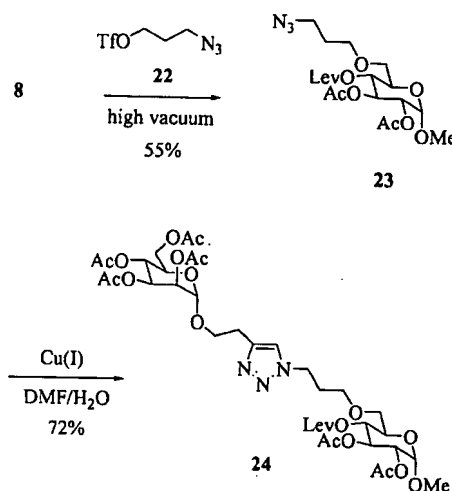
Scheme 6.

Table 2. Structures of the cycloadducts obtained and experimental conditions.

Entry	Product	CuSO4	Na asc.	Time	Yield
1		0.2 equiv.	0.4 equiv.	1.5 h	14 a-Man 88%
2		0.4 equiv.	0.8 equiv.	1.6 h	16 a-Gal 76%
3		0.2 equiv.	0.4 equiv.		17 ß-Glc 75%
4	18	0.4 equiv.	0.8 equiv.	5 h	70%
5	19	0.2 equiv.	0.4 equiv.	2.5 h	100%
6	20	0.2 equiv.	0.4 equiv.	3 h	100%
7	21	0.4 equiv.	0.8 equiv.	1.1 h	75%

Conclusion

In summary, we have developed a procedure for the connection of saccharides by click chemistry techniques. This approach shows that different sugars bearing azide substituents on C-2, C-5, or C-6 can be used as building blocks. The reactions tolerate the presence of a range of protecting groups commonly used in carbohydrate chemistry. Moreover, they can be conducted at room temperature, with equimolar ratios of reagents, and products are obtained in high yields. The high solubilities of the protected sugars in DMF allowed us to add 30% water to the mixtures, which greatly accelerated the cycloaddition reactions. Furthermore, we have also demonstrated the possibility of varying the distances between triazole-linked saccharides units, through the use of spacers to attach the azido group.

Experimental Section

General: All purchased materials were used without further purification. Dichloromethane was distilled from calcium hydride, tetrahydrofuran over sodium and benzophenone. Analytical thin-layer chromatography (TLC) was carried out on Merck D.C.-Alufolien (Kieselgel 60 F_{254}). Flash chromatography was performed on Geduran Si 60 (0.040–0.060 mm pore size) with distilled solvents. ^{1}H, ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 300 spectrometer, and chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent (CHCl$_3$) peak (^{1}H: $\delta = 7.26$ ppm, ^{13}C: $\delta = 77.2$ ppm). Assignments. of ^{1}H and ^{13}C were assisted by 2D ^{1}H COSY and 2D ^{1}H–^{13}C CORR experiments. High resolution mass spectra HRMS were obtained by Electrospray Ionization (ESI) with a Micromass–Waters Q-TOF Ultima Global instrument. Optical rotations were measured on a Perkin–Elmer 343 machine at 20 °C in a 1-cm cell in the

stated solvent. $[a]_D$ values are given in 10^{-1} deg cm^2 g^{-1} (concentration c given as g/100 mL).

Phenyl 3-Acetyl-2-azido-2-deoxy-4,6-O-benzylidene-1-thio-α-D-glucopyranoside (3): The known phenyl 2-azido-4,6-O-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside[16] (1.49 g, 3.9 mmol) was dissolved in a mixture of acetic anhydride (20 mL) and pyridine (25 mL) and the mixture was stirred at room temp. for 18 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (cyclohexane/EtOAc, 9:1) to afford **3** as a white solid (1.6 g, 96%). $[a]_D^{20}$ = +145 (c = 1, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.44 (m, 10 H, arom. H), 5.65 (d, $J_{1,2}$ = 6.0 Hz, 1 H, 1-H), 5.54 (s, 1 H, PhCH), 5.53 (dd, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, 1 H, 3-H), 4.51 (ddd, $J_{4,5}$ = 10.0, $J_{5,6a}$ = 5.0, $J_{5,6b}$ = 10.0 Hz, 1 H, 5-H), 4.26 (dd, $J_{6a,6b}$ = 10.0 Hz, 1 H, 6a-H), 4.07 (dd, 1 H, 2-H), 3.80 (dd, 1 H, 6b-H), 3.70 (dd, 1 H, 4-H), 2.18 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 169.9 (CO), 137.2, 134.2, 133.1, 128.6, 126.6 (arom. C), 102.1 (PhCH), 88.2 (C-1), 80.0 (C-4), 70.9 (C-3), 68.8 (C-6), 64.3 (C-5), 62.7 (C-2), 21.3 (CH_3) ppm. HRMS: m/z: found 450.1114; C$_{21}$H$_{21}$O$_5$N$_3$NaS requires 450.1100 [M + Na]$^+$.

Methyl 2,3-Di-O-acetyl-6-O-benzyl-4-O-levulinyl-α-D-glucopyranoside (7): Levulinic anhydride (1.8 g, 8.41 mmol) in dichloromethane (5 mL), Et$_3$N (2.36 mL, 16.8 mmol), and DMAP (25 mg, 0.20 mmol) were added under argon to a solution of compound **6** (1.55 g, 4.21 mmol) in dry dichloromethane (20 mL), and the mixture was stirred for 16 h at room temp. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (cyclohexane/EtOAc, 7:3 to 13:7) to afford **7** as a colorless oil (1.91 g, 97%). $[a]_D^{20}$ = +118 (c = 0.2, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.30 (m, 5 H, arom. H), 5.49 (dd, $J_{2,3}$ = 10.0, $J_{3,4}$ = 9.5 Hz, 1 H, 3-H), 5.15 (dd, $J_{4,5}$ = 10.0 Hz, 1 H, 4-H), 4.93 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 4.91 (dd, 1 H, 2-H), 4.54 (s, 2 H, CH_2Ph), 3.94 (m, 1 H, 5-H), 3.56 (m, 2 H, 6-a, 6b-H), 3.42 (s, 3 H, CH_3O), 2.67 (t, J = 6.3 Hz, 2 H, CH$_3$COCH_2CH$_2$), 2.40 (t, J = 6.3 Hz, 2 H, CH$_3$COCH$_2$CH_2), 2.15 (s, 3 H, CH_3Lev), 2.07, 2.04 (2×s, 3 H each, 2×CH_3CO) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 206.1 (CH$_3$COCH$_2$), 171.8, 170.7 (CH$_3$CO), 170.6 (COCH$_2$CH$_2$), 138.2, 128.7, 128.3, 128.1 (arom. C), 97.1 (C-1), 74.0 (PhCH_2), 71.4 (C-2), 70.4 (C-3), 69.6 (C-4), 68.8 (C-5, -6), 55.8 (CH_3O), 38.1 (CH$_3$COCH_2CH$_2$), 29.1 (CH_3Lev), 28.2 (CH$_3$COCH$_2$$CH_2$), 21.1 ($CH_3$CO) ppm. HRMS: m/z: found 489.1715; C$_{23}$H$_{30}$NaO$_{10}$ requires 489.1737 [M + Na]$^+$.

Methyl 2,3-Di-O-acetyl-4-O-levulinyl-α-D-glucopyranoside (8): A mixture of compound **7** (200 mg, 0.429 mmol) and Pd/C (10%, 20 mg) in THF (6 mL) was stirred for 40 min at room temp. under a positive pressure of hydrogen. The mixture was filtered through celite, and the solvent was removed under reduced pressure to give **7** as a colorless oil (161 mg, quant). $[a]_D^{20}$ = +94 (c = 0.2, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 5.55 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, 1 H, 3-H), 5.03 (dd, $J_{4,5}$ 9.8 Hz, 1 H, 4-H), 4.96 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 4.86 (dd, 1 H, 2-H), 3.78–3.62 (m, 3 H, 5-, 6a-, 6b-H), 3.40 (s, 3 H, CH_3O), 2.74 (m, 2 H, CH$_3$COCH_2CH$_2$), 2.50 (m, 2 H, CH$_3$COCH$_2$CH_2), 2.17 (s, 3 H, CH_3Lev), 2.07, 2.04 (2×s, 3 H each, CH_3CO) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 172.9, 170.6 (CO), 97.2 (C-1), 71.5 (C-2), 70.1, 69.9, 69.8 (C-3, -4, -5), 61.3 (C-6), 55.8 (CH_3O), 38.1 (CH$_3$COCH_2CH$_2$), 30.0 (CH_3Lev), 28.2 (CH$_3$COCH$_2$$CH_2$), 21.1 ($CH_3$CO) ppm. HRMS: m/z: found 399.1256; C$_{16}$H$_{24}$NaO$_{10}$ requires 399.1267 [M + Na]$^+$.

Methyl 2,3-Di-O-acetyl-6-O-iodo-4-O-levulinyl-α-D-glucopyranoside (9): A solution of compound **8** (3.3 g, 8.8 mmol), PPh$_3$ (6.9 g, 26 mmol), imidazole (2.38 g, 35 mmol), and I$_2$ (5.57 g, 21.9 mmol) in anhydrous toluene (160 mL) was stirred at room temp. for 6 h.

The reaction mixture was cooled and satd. NaHCO$_3$ (160 mL) was added. After the mixture had been stirred for 5 min, I$_2$ (16.5 g, 65 mmol) was added and after an additional 10 min excess iodine was removed by addition of a sodium thiosulfate solution (100 mL). The organic layer was diluted with toluene, separated, washed with water, dried with Na$_2$SO$_4$, and filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (cyclohexane/EtOAc, 3:1) to afford **9** as a colorless oil (2.89 g, 67%). $[a]_D^{20}$ = +73 (c = 0.3, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 5.47 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, 1 H, 3-H), 4.94 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 4.88 (dd, $J_{4,5}$ = 9.5 Hz, 1 H, 4-H), 4.82 (dd, 1 H, 2-H), 3.77 (m, 1 H, 5-H), 3.46 (s, 3 H, CH_3O), 3.60 (dd, $J_{5,6a}$ = 2.4, $J_{6a,6b}$ = 10.9 Hz, 1 H, 6a-H), 3.60 (dd, $J_{5,6b}$ = 8.4 Hz, 1 H, 6b-H), 2.72 (m, 2 H, CH$_3$COCH_2CH$_2$), 2.49 (m, 2 H, CH$_3$COCH$_2$CH_2), 2.17 (s, 3 H, CH_3Lev), 2.06, 2.02 (2×s, 3 H each, 2×CH$_3$CO) ppm. ^{13}C NMR (75 MHz CDCl$_3$): δ = 206.2 (CH$_3$CO), 171.6, 170.1 (CO), 96.8(C-1), 72.5, 71.1 (C-2, C-4), 69.4 (C-3), 68.9 (C-5), 55.8 (CH_3O), 38.9 (CH$_3$COCH$_2$CH$_2$), 30.8 (CH_3Lev), 27.1 (CH$_3$COCH$_2$CH_2), 20.8 (CH_3CO), 3.9 (C-6) ppm. HRMS: m/z: found 509.0294; C$_{16}$H$_{23}$INaO$_9$ requires 509.0285 [M + Na]$^+$.

Methyl 2,3-Di-O-acetyl-6-azido-6-deoxy-4-O-levulinyl-α-D-glucopyranoside (4): A mixture of compound **9** (150 mg, 0.3 mmol) and LiN$_3$ (20% in water, 377 µL, 1.5 mmol) in dry DMF (5 mL) was stirred under argon at 50 °C until total consumption of starting material. EtOAc (20 mL) was added and the organic layer was washed with water (20 mL). The aqueous layer was extracted three times with EtOAc (3 × 10 mL). The organic layers were combined, dried with Na$_2$SO$_4$, and filtered, and the solvent was removed under reduced pressure to afford **4** (90 mg, 75%). $[a]_D^{20}$ = +107 (c = 0.2, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 5.56 (dd, $J_{2,3}$ = 10, $J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 5.09 (dd, $J_{4,5}$ = 9.5 Hz, 1 H, 4-H), 5.04 (d, $J_{1,2}$ = 3.7 Hz, 1 H, 1-H), 4.94 (dd, 1 H, 2-H), 4.02 (dt, $J_{5,6a}$ = $J_{5,6b}$ = 5.0 Hz, 1 H, 5-H), 3.52 (s, 3 H, CH_3O), 3.44 (d, 2 H, 6a-, 6b-H), 2.80 (m, 2 H, CH$_3$COCH_2CH$_2$), 2.54 (m, 2 H, CH$_3$COCH$_2$CH_2), 2.24 (s, 3 H, CH_3Lev), 2.15, 2.12 (2×s, 3 H each, 2×CH$_3$CO) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 206.4 (CH$_3$CO), 171.7, 170.3, 170.2, (CO), 96.7(C-1), 70.9 (C-2), 69.7, 69.5 (C-3, -4), 68.7 (C-5), 55.6 (CH_3O), 50.9 (C-6), 38.9 (CH$_3$COCH_2CH$_2$), 30.8 (CH_3Lev), 27.8 (CH$_3$COCH$_2$CH_2), 20.8 (CH_3CO) ppm. HRMS: m/z: found 424.1346; C$_{16}$H$_{23}$N$_3$NaO$_9$ requires 424.1332 [M + Na]$^+$.

But-3-ynyl 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranoside (11): This compound was obtained by the procedure described in ref.[22]. The pure α anomer was separated from the α/β mixture by flash chromatography (dichloromethane/EtOAc, 98:2, 20% yield). $[a]_D^{20}$ = +130 (c = 0.1, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 5.43 (br d, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ < 1 Hz, 1 H, 4-H), 5.33 (dd, $J_{2,3}$ = 11.0 Hz, 1 H, 3-H), 5.15 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1-H), 5.09 (dd, 1 H, 2-H), 4.28 (t, $J_{5,6a}$ = $J_{5,6b}$ = 7.0 Hz, 1 H, 5-H), 4.09–4.04 (m, 2 H, 6a-, 6b-H), 3.75 (dt, J = 7, J = 10 Hz, 1 H, OCHHCH$_2$), 3.60 (dt, J = 7, J = 10 Hz, 1 H, OCHHCH$_2$), 2.47 (dt, J = 3, J = 7 Hz, 2 H, OCH$_2$CH_2), 2.11, 2.05, 2.02, 1.97 (4×s, 3 H each, 4×CH_3CO), 1.95 (m, 1 H, CCH) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 170.5, 170.3, 170.0 (CO), 96.3 (C-1), 80.8 (CH$_2$$C$CH), 69.8 (CH$_2CCH$), 68.2 (C-2, -4), 67.6 (C-3), 66.7 (OCH_2CH$_2$CCH), 66.6 (C-5), 61.8 (C-6), 20.9, 20.7 (CH_3CO), 19.8 (OCH$_2$$CH_2$) ppm. HRMS: m/z: found 423.1282; C$_{18}$H$_{24}$NaO$_{10}$ requires 423.1267 [M + Na]$^+$.

But-3-ynyl 2,3,4,6,2′,3′,6′-Hepta-O-acetyl-β-D-lactopyranoside (13): This compound was obtained by the procedure described in ref.[22]. The pure β product was isolated by flash chromatography (cyclohexane/EtOAc, 2:3 to 1:1, 43% yield). $[a]_D^{20}$ = –9 (c = 0.4, CH$_2$Cl$_2$).

^{1}H NMR (300 MHz, CDCl$_3$): δ = 5.31 (br d, $J_{3',4'}$ = 3 Hz, $J_{4',5'}$ < 1 Hz, 1 H, 4'-H), 5.13 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1 H, 3-H), 5.06 (dd, $J_{1',2'}$ = $J_{2',3'}$ = 8.0 Hz, 1 H, 2'-H), 4.90 (dd, 1 H, 3'-H), 4.85 (dd, $J_{1,2}$ = 8 Hz, 1 H, 2-H), 4.50 (d, 1 H, 1-H), 4.50 (m, 2 H, 1'-, 6a-H), 4.09–4.03 (m, 4 H, 6b-, 5'-, 6'a- 6'b-H), 3.88–3.75 (m, 2 H, 5'-H, OC*H*HCH$_2$), 3.74 (dd, 1 H, $J_{4,5}$ = 9 Hz, 4-H), 3.63–3.59 (m, 2 H, 5-H, OCH*H*CH$_2$), 2.42 (dt, 2 H, J = 3.0, J = 7.0 Hz, OCH$_2$C*H$_2$*), 2.11, 2.08 (×2), 2.02, 2.01(×2), 1.92 (7 × s, 3 H each, 7 × C*H$_3$*CO), 1.92 (m, 1 H, CC*H*) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 170.7, 170.5, 170.4, 170.1, 170.0, 169.4 (*C*O), 101.4 (C-1'), 101.0 (C-1), 80.9 (*C*CH), 76.6 (C-4), 73.0 (C-3), 71.9 (C-2), 71.4 (C-3'), 71.0 (C-5'), 69.9 (CH$_2$CCH), 69.5 (C-2'), 68.3 (O*C*H$_2$CH$_2$CCH), 67.0 (C-4'), 62.3, 61.2 (C-6, -6'), 21.1, 21.1, 21.0, 20.9 (*C*H$_3$CO), 20.2 (OCH$_2$*C*H$_2$) ppm. HRMS: *m/z*: found 711.2089; C$_{30}$H$_{40}$NaO$_{18}$ requires 711.2112 [M + Na]$^+$.

General Procedure for the Huisgen Cycloadditions: Compounds 14–21 and 24 were synthesized by this procedure. Alkynyl-saccharide (0.13 mmol) and azido-saccharide (0.13 mmol) were dissolved in a DMF/H$_2$O mixture (4+1.3 mL). Copper sulfate (0.2 or 0.4 equiv.) and sodium ascorbate (0.4 or 0.8 equiv.) were added and the mixture was stirred at room temp. until TLC indicated the disappearance of the starting materials. The mixture was poured into H$_2$O/ satd. NH$_4$Cl solution (1:1, 20 mL) and the product was extracted four times with EtOAc. The organic layer was dried with Na$_2$SO$_4$ and filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography.

1-(2',3',4',6'-Tetra-O-acetyl-α-D-mannopyranosyloxyethyl)-4-(methyl 2'',3''-di-O-acetyl-6''-deoxy-4''-O-levulinyl-α-D-glucopyranosid-6-yl)-1H-1,2,3-triazole (14): Flash chromatography (EtOAc/cyclohexane, 4:1 to EtOAc). [a]$_D^{20}$ = +80 (*c* = 0.4, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.55 (s, 1 H, CH*N*), 5.46 (dd, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, 1 H, 3-H), 5.27–5.15 (m, 5 H, 2'-, 3'-, 4'-H), 4.89 (d, $J_{1',2'}$ = 3.0 Hz, 1 H, 1'-H), 4.86–4.79 (m, 3 H, 1-, 2-, 4-H), 4.59 (br d, $J_{5,6a}$ < 1, $J_{6a,6b}$ = 13.9 Hz, 1 H, 6a-H), 4.50–3.90 (m, 5 H, 5-, 6b-, 6'a-, 6'b-H, OC*H*HCH$_2$), 3.80–3.73 (m, 2 H, 5'-H, OCH*H*CH$_2$), 3.09 (s, 3 H, C*H$_3$*O), 3.05–3.01 (m, 2 H, OCH$_2$CH$_2$), 2.75 (m, 2 H, CH$_3$COC*H$_2$*CH$_2$), 2.52 (m, 2 H, CH$_3$COCH$_2$C*H$_2$*), 2.15 (s, 3 H, C*H$_3$*Lev), 2.14, 2.12, 2.06, 2.02, 2.01, 1.97 (6 × s, 3 H each, 6 × C*H$_3$*CO) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 206.7 (CH$_3$*C*O), 171.9, 170.8, 170.2, 169.7 (*C*O), 144.2 (N*C*=CH), 123.8 (NC=*C*H), 97.5 (C-1'), 96.6 (C-1), 70.8 (C-2), 70.2 (C-4), 69.6, 69.5, 66.1 (C-2', -3', -4'), 69.1 (C-5'), 68.8 (C-5), 68.1 (C-3'), 67.0 (O*C*H$_2$CH$_2$), 62.4 (C-6'), 55.3 (*C*H$_3$O), 50.6 (C-6), 37.9 (CH$_3$CO*C*H$_2$CH$_2$), 29.7 (*C*H$_3$Lev), 28.0 (CH$_3$COCH$_2$*C*H$_2$), 26.2 (O*C*H$_2$CH$_2$), 20.9 (*C*H$_3$CO) ppm. HRMS: *m/z*: found 824.2686; C$_{34}$H$_{47}$N$_3$NaO$_{19}$ requires 824.2701 [M + Na]$^+$.

1-(α-D-Mannopyranosyloxyethyl)-4-(methyl 6''-deoxy-α-D-glucopyranosid-6-yl)-1H-1,2,3-triazole (15): Flash chromatography (EtOAc/cyclohexane, 4:1 to EtOAc). [a]$_D^{20}$ = +59 (*c* = 0.1, D$_2$O). ^{1}H NMR (300 MHz, CD$_3$OD): δ = 7.26 (s, 1 H, CH*N*), 4.28 (dd, $J_{5,6a}$ = 7.7, $J_{6a,6b}$ = 14.2 Hz, 1 H, 6a-H), 4.19 (d, $J_{1',2'}$ = 2.0 Hz, 1 H, 1'-H), 4.10 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1-H), 3.98 (dd, 1 H, $J_{5,6b}$ = 8.0 Hz, 6b-H), 3.42–3.36 (m, 1 H, OCH*H*CH$_2$), 3.30–2.50 (m, 11 H, 2-, 3-, 4-, 5-, 2'-, 3'-, 4'-, 5'-, 6'a-, 6'b-H, OCH*H*CH$_2$), 2.60 (s, 3 H, C*H$_3$*O), 2.43 (m, 2 H, OCH$_2$C*H$_2$*) ppm. ^{13}C NMR (75 MHz, CD$_3$OD): δ = 146.0 (N*C*=CH), 125.5 (NC=*C*H), 101.6 (C-1'), 101.2 (C-1), 74.9, 74.8, 73.3, 72.8, 72.5, 72.0, 71.6, 68.5 (C-2, -3, -4, -5, -2', -3', -4', -5'), 67.4 (O*C*H$_2$CH$_2$), 62.9 (C-6'), 55.3 (*C*H$_3$O), 52.3 (C-6), 27.0 (OCH$_2$*C*H$_2$) ppm. HRMS: *m/z*: found 474.1704; C$_{17}$H$_{29}$N$_3$NaO$_{11}$ requires 474.1700 [M + Na]$^+$.

1-(2',3',4',6'-Tetra-O-acetyl-α-D-galactopyranosyloxyethyl)-4-(methyl 2'',3''-di-O-acetyl-6''-deoxy-4''-O-levulinyl-α-D-glucopyr-

anosid-6-yl)-1H-1,2,3-triazole (16): Flash chromatography (EtOAc/cyclohexane, 4:1 to EtOAc). [a]$_D^{20}$ = +110 (*c* = 0.2, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.50 (s, 1 H, CH*N*), 5.44 (dd, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, 1 H, 3-H), 5.43 (br d, $J_{3',4'}$ = 3.0, $J_{4,5}$ < 1 Hz, 1 H, 4'-H), 5.26 (dd, $J_{2',3'}$ = 11, $J_{3',4'}$ = 3.0 Hz, 1 H, 3'-H), 5.09 (d, $J_{1',2'}$ = 4.0 Hz, 1 H, 1'-H), 5.08 (dd, $J_{1',2'}$ = 4.0 Hz, 1 H, 2'-H), 4.79 (m, 3 H, 1-, 2-, 4-H), 4.59 (dd, $J_{5,6a}$ = 2.2, $J_{6a,6b}$ = 14.3 Hz, 1 H, 6a-H), 4.25 (dd, $J_{5,6b}$ = 8.3 Hz, 1 H, 6b-H), 4.20–3.90 (m, 5 H, 5-, 5'-, 6'a-, 6'b-, OC*H*HCH$_2$), 3.75 (dt, J = 7.0, J = 10.0 Hz, 1 H, OCH*H*CH$_2$), 3.08 (s, 3 H, C*H$_3$*O), 3.02 (t, J = 7.0 Hz, 2 H, OCH$_2$C*H$_2$*), 2.73 (m, 2 H, CH$_3$COC*H$_2$*CH$_2$), 2.50 (m, 2 H, CH$_3$COCH$_2$C*H$_2$*), 2.14 (s, 3 H, C*H$_3$*Lev), 2.09 (×2), 1.99, 1.95 (×2), 1.93 (6 × s, 3 H each, 6 × C*H$_3$*CO) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 206.7 (CH$_3$*C*O), 171.8, 170.5, 170.3, 170.1 (*C*O), 144.3 (N*C*=CH), 123.3 (NC=*C*H), 97.5 (C-1'), 96.6 (C-1), 96.2 (C-1'), 70.8 (C-2), 70.0 (C-4), 69.4 (C-3), 68.1 (C-2', -4'), 66.4 (C-5'), 67.5 (C-3'), 67.4 (O*C*H$_2$CH$_2$), 66.4 (C-5), 62.1 (C-6'), 55.7 (*C*H$_3$O), 50.8 (C-6), 38.2 (CH$_3$CO*C*H$_2$CH$_2$), 29.9 (C*H$_3$*Lev), 28.3 (CH$_3$COCH$_2$*C*H$_2$), 26.5 (O*C*H$_2$CH$_2$), 21.0 (*C*H$_3$CO) ppm. HRMS: *m/z*: found 824.2682; C$_{34}$H$_{47}$N$_3$NaO$_{19}$ requires 824.2701 [M + Na]$^+$.

1-(2',3',4',6'-Tetra-O-acetyl-β-D-glucopyranosyloxyethyl)-4-(methyl 2'',3''-di-O-acetyl-6''-deoxy-4''-O-levulinyl-α-D-glucopyranosid-6-yl)-1H-1,2,3-triazole (17): Flash chromatography (EtOAc/cyclohexane, 4:1 to EtOAc). [a]$_D^{20}$ = +32 (*c* = 0.3, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.49 (s, 1 H, CH*N*), 5.45 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1 H, 3-H), 5.13 (dd, $J_{2',3'}$ = $J_{3',4'}$ = 10.0 Hz, 1 H, 3'-H), 5.13 (dd, $J_{3',4'}$ = $J_{4',5'}$ = 10.0 Hz, 1 H, 4'-H), 5.12–4.79 (m, 4 H, 1-, 2-, 2'-, 4-H), 4.59 (dd, $J_{5,6a}$ = 2.2, $J_{6a,6b}$ = 14.2 Hz, 1 H, 6a-H), 4.50 (d, $J_{1',2'}$ = 8.0 Hz, 1 H, 1'-H), 4.35–4.00 (m, 5 H, 5-, 6b-, 6'a-, 6'b-H, OC*H*HCH$_2$), 3.75 (m, 2 H, 5'-H, OCH*H*CH$_2$), 3.10 (s, 3 H, C*H$_3$*O), 2.93 (t, 2 H, J = 7.0 Hz, OCH$_2$C*H$_2$*), 2.75 (m, 2 H, CH$_3$COC*H$_2$*CH$_2$), 2.50 (m, 2 H, CH$_3$COCH$_2$C*H$_2$*), 2.15 (s, 3 H, C*H$_3$*Lev), 2.09, 2.05, 2.03, 1.98, 1.96, 1.95 (6 × s, 3 H each, 6 × C*H$_3$*CO) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 206.7 (CH$_3$*C*O), 171.9, 170.7, 170.2, 169.5, 169.4 (*C*O), 144.4 (N*C*=CH), 123.8 (NC=*C*H), 100.9 (C-1'), 96.6 (C-1), 72.8 (C-3'), 71.9 (C-5'), 71.3 (C-2'), 70.8 (C-2), 70.0 (C-4), 69.5 (C-3), 69.0 (O*C*H$_2$CH$_2$), 68.5 (C-4'), 68.0 (C-5), 61.2 (C-6'), 55.5 (*C*H$_3$O), 50.5 (C-6), 37.9 (CH$_3$CO*C*H$_2$CH$_2$), 29.7 (*C*H$_3$Lev), 28.0 (CH$_3$COCH$_2$*C*H$_2$), 26.5 (O*C*H$_2$CH$_2$), 20.8 (*C*H$_3$CO) ppm. HRMS: *m/z*: found 824.2695; C$_{34}$H$_{47}$N$_3$NaO$_{19}$ requires 824.2701 [M + Na]$^+$.

1-(2',3',4',6',2'',3'',6''-Hepta-O-acetyl-β-D-lactopyranosyloxyethyl)-4-(methyl 2''',3'''-di-O-acetyl-6''-deoxy-4''-O-levulinyl-α-D-glucopyranosid-6-yl)-1H-1,2,3-triazole (18): Flash chromatography (EtOAc/cyclohexane, 4:1 to EtOAc). [a]$_D^{20}$ = +13 (*c* = 0.3, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.49 (s, 1 H, CH*N*), 5.43 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1 H, 3-H), 5.30 (br d, 1 H, $J_{3'',4''}$ = 3.0, $J_{4'',5''}$ < 1 Hz, 4''-H), 5.13 (dd, $J_{2',3'}$ = $J_{3',4'}$ = 9.0 Hz, 1 H, 3'-H), 5.06 (dd, $J_{1'',2''}$ = $J_{2',3'}$ = 8.0 Hz, 1 H, 2''-H), 4.90–4.78 (m, 5 H, 1-, 2-, 2'-, 3''-, 4-H), 4.59 (dd, $J_{5,6a}$ = 2.3, $J_{6a,6b}$ = 14.3 Hz, 1 H, 6a-H), 4.44 (m, 3 H, 1'-, 1''-, 6'a-H), 4.25 (dd, $J_{5,6b}$ = 8.4 Hz, 1 H, 6b-H), 4.20–4.00 (m, 5 H, 5-, 6'b-, 6''a-, 6''b-H, OC*H*HCH$_2$), 3.80 (m, 1 H, 5''-H), 3.74 (m, 2 H, 4'-H, OCH*H*CH$_2$), 3.52 (m, 1 H, 5'-H), 3.07 (s, 3 H, C*H$_3$*O), 2.91 (t, J = 7.0 Hz, 2 H, OCH$_2$C*H$_2$*), 2.72 (m, 2 H, CH$_3$COC*H$_2$*CH$_2$), 2.50 (m, 2 H, CH$_3$COCH$_2$C*H$_2$*), 2.14 (s, 3 H, C*H$_3$*Lev), 2.10, 2.08, 2.01 (×2), 1.98, 1.97 (×2), 1.93, 1.91 (9 × s, 3 H each, 9 × C*H$_3$*CO) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 206.7 (CH$_3$*C*O), 172, 170.7, 170.5, 170.4, 170.0, 169.5 (*C*O), 144.8 (N*C*=CH), 124.0 (NC=*C*H), 101.4 (C-1''), 101.0 (C-1'), 96.9 (C-1), 76.7 (C-4'), 72.7 (C-3', -5'), 71.6 (C-2'), 71.0, 70.8, 70.7, 70.0 (C-2, -4, -3'', -5''), 69.5 (C-3), 69.2 (C-2''), 69.1 (O*C*H$_2$CH$_2$), 68.9 (C-5), 67.0 (C-4''), 62.3, 61.2 (C-6', -6''), 55.7

(CH_3O), 50.8 (C-6), 38.1 ($CH_3COCH_2CH_2$), 29.7 (CH_3Lev), 28.3 ($CH_3COCH_2CH_2$), 26.7 (OCH_2CH_2), 21.0 (CH_3CO) ppm. HRMS: m/z: found 1112.3516; $C_{46}H_{63}N_3NaO_{27}$ requires 1112.3547 [M + Na]$^+$.

1-(2′,3′,4′,6′-Tetra-O-acetyl-α-D-mannopyranosyloxyethyl)-4-(6-azido-1′′,2′′:3′′,4′′-di-O-isopropylidene-6-deoxy-α-D-galactopyrano-sid-6-yl)-1H-1,2,3-triazole (19): Flash chromatography (EtOAc/cyclohexane, 4:1 to EtOAc). [$α$]$_D^{20}$ = −3 (c = 0.3, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): $δ$ = 7.63 (s, 1 H, CHN), 5.46 (d, $J_{1,2}$ = 5.0 Hz, 1 H, 1-H), 5.25–5.16 (m, 3 H, 2′-, 3′-, 4′-H), 4.79 (s, 1 H, 1′-H), 4.59 (dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 8.0 Hz, 1 H, 3-H), 4.54 (dd, $J_{5,6a}$ = 4.0, $J_{6a,6b}$ = 14.0 Hz, 1 H, 6a-H), 4.27 (dd, $J_{5,6b}$ = 8.0 Hz, 1 H, 6b-H), 4.27 (dd, 1 H, 2′-H), 4.23–4.04 (m, 5 H, 4-, 5-, 6′a-, 6′b-H, OCHHCH$_2$), 4.00 (dt, J = 6.0, J = 10.0 Hz, 1 H, OCHHCH$_2$), 3.80 (m, 1 H, 5′-H), 3.67 (dt, J = 6.0, J = 10.0 Hz, 1 H, OCHHCH$_2$), 3.01 (t, J = 7.0 Hz, 2 H, OCH$_2$CH_2), 2.12, 2.05, 1.99, 1.94 (4 × s, 3 H each, 4 × CH_3CO), 1.45, 1.33, 1.31, 1.23 (4 × s, 3 H each, 4 × CH_3C) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): $δ$ = 170.7, 170.0, 169.9, 169.7 (CO), 143.9 (NC=CH), 123.2 (NC=CH), 97.5 (C-1′), 96.6 (C-1), 71.3 (C-5′), 70.8 (C-3), 70.4 (C-2), 69.6, 69.1, 66.1 (C-4, -2′, -3′, -4′), 68.6 (C-5′), 67.3 (C-5), 67.2 (OCH$_2$CH$_2$), 62.4 (C-6′), 50.5 (C-6), 28.0 ($CH_3COCH_2CH_2$), 26.2 (OCH$_2$CH$_2$), 26.0, 25.9, 24.9, 24.5 (CH$_3$C), 20.9, 20.7 (CH$_3$CO) ppm. HRMS: m/z: found 708.2618; $C_{30}H_{43}N_3NaO_{15}$ requires 708.2592 [M + Na]$^+$.

2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyloxyethyl)-4-(phenyl 2′′-deoxy-4,6-O-benzylidene-1-thio-α-D-glucopyranosid-2-yl)-1H-1,2,3-triazole (20): Flash chromatography (dichloromethane, then EtOAc). [$α$]$_D^{20}$ = +48 (c = 0.4, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): $δ$ = 7.49 (s, 1 H, CHN), 7.44–7.22 (m, 10 H, arom. H), 5.95 (dd, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, 1 H, 3-H), 5.77 (d, $J_{1,2}$ = 5.0 Hz, 1 H, 1-H), 5.55 (s, 1 H, PhCH), 5.34 (dd, 1 H, 2-H), 5.17 (dd, $J_{2′,3′}$ = $J_{3′,4′}$ = 10.0 Hz, 1 H, 3′-H), 5.08 (dd, $J_{4′,5′}$ = 10.0 Hz, 1 H, 4′-H), 4.97 (dd, $J_{1′,2′}$ = 10.0 Hz, 1 H, 2′-H), 4.54–4.49 (m, 2 H, 1′-, 5-H), 4.30–4.27 (m, 2 H, 6a-, 6′a-H), 4.02–3.98 (m, 2 H, 6′-H, OCHHCH$_2$), 3.88–3.85 (m, 3 H, 4-, 6-H, OCHHCH$_2$), 3.72–3.69 (m, 1 H, 5′-H), 3.02 (t, J = 7 Hz, 2 H, OCH$_2$CH_2), 2.07, 2.05, 2.04 (× 2), 1.99, 1.97 (6 × s, 3 H each, 6 × CH_3CO) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): $δ$ = 170.5, 170.1, 169.7, 169.2, 169.1 (CO), 144.0 (NC=CH), 137.5, 134.0, 133.3, 128.9, 128.2, 126.0 (arom. C), 121.5 (NC=CH), 101.6 (PhCH), 100.5 (C-1′), 88.2 (C-1), 79.8 (C-4), 72.7 (C-3′), 71.7 (C-5′), 71.3 (C-2′), 70.9 (C-3), 69.0 (OCH$_2$CH$_2$), 68.6 (C-6), 68.2 (C-4′), 64.3 (C-5), 62.9 (C-2), 61.8 (C-6′), 26.3 (OCH$_2$$CH_2$), 20.4 ($CH_3$CO) ppm. HRMS: m/z: found 850.2477; $C_{39}H_{45}N_3NaO_{15}$S requires 850.2469 [M + Na]$^+$.

2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyloxyethyl)-4-(5-azido-5-deoxy-1,2-O-isopropylidene-3,6-di-O-benzyl-β-L-idofuranosid-5-yl)-1H-1,2,3-triazole (21): Flash chromatography (EtOAc). [$α$]$_D^{20}$ = −24 (c = 0.2, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): $δ$ = 7.52 (s, 1 H, CHN), 7.34–7.19 (m, 10 H, arom. H), 5.88 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1-H), 5.18 (dd, $J_{2′,3′}$ = $J_{3′,4′}$ = 9.0 Hz, 1 H, 3′-H), 5.02 (dd, $J_{4′,5′}$ = 9.0 Hz, 1 H, 4′-H), 4.99 (m, 2 H, 2′-, 5-H), 4.62 (dd, $J_{3,4}$ = 4.0, $J_{4,5}$ = 13.0 Hz, 1 H, 4-H), 4.59 (dd, $J_{2,3}$ = 3.0 Hz, 1 H, 2-H), 4.56 (m, 2 H, 1′-H, PhCH), 4.39 (d, J = 12.1 Hz, 1 H, PhCH), 4.30–4.22 (m, 3 H, 6′-H, 2 PhCH), 4.13–3.94 (m, 2 H, 6′-H, OCHHCH$_2$), 3.94 (d, 1 H, 3-H), 3.90–3.60 (m, 4 H, OCHHCH$_2$, 5′-, 6a-, 6b-H), 3.01 (t, J = 7.0 Hz, 2 H, OCH$_2$CH_2), 2.06, 2.02, 1.99, 1.97 (4 × s, 3 H each, 4 × CH_3CO) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): $δ$ = 170.8, 170.3, 169.5 (CO), 143.5 (NC=CH), 137.3, 136.7, 128.7, 128.5, 128.3 (arom. C), 122.7 (NC=CH), 112.0 (CH$_3$$CCH_3$), 104.8 (C-1), 100.9 (C-1′), 81.8 (C-2), 81.3 (C-3), 78.7 (C-4), 73.4 (PhCH$_2$), 72.9 (C-3′), 71.8 (C-5′), 71.7 (PhCH$_2$), 71.3 (C-2′), 69.1 (OCH$_2$CH$_2$), 68.7 (C-6), 68.5 (C-4′), 62.0 (C-6′), 60.5 (C-5), 26.8 (CH$_3$C), 26.5

(OCH_2CH_2), 26.3 (CH_3C), 20.8 (CH_3CO) ppm. HRMS: m/z: found 848.3218; $C_{41}H_{51}N_3NaO_{15}$ requires 848.3218 [M + Na]$^+$.

Methyl 2,3-Di-O-acetyl-6-O-azidopropyl-4-O-levulinyl-α-D-glucopyranoside (23): 3-Azidopropan-1-ol (691 mg, 6.84 mmol, prepared from 3-chloropropan-1-ol as in ref.[29]) and 2,6-di-*tert*-butyl-4-methylpyridine (1.4 g, 6.84 mmol) were dissolved in dry dichloromethane (37 mL). Tf$_2$O (1.135 mL, 6.84 mmol) was added at 0 °C and the mixture was stirred at room temp. for 15 min. Water (100 mL) was added and the product **22** was extracted with CH$_2$Cl$_2$ (2 × 150 mL). The organic layer was dried with MgSO$_4$ and concentrated to 310 mL (c = 22.5 mM). Compound **8** (507 mg, 1.35 mmol), was added to the solution (60 mL) and the solvent was removed on a rotavapor and under high vacuum. The procedure was repeated three times. The residue was purified by flash chromatography (cyclohexane/EtOAc, 3:1 to 1:1) to afford **23** as a colorless oil (340 mg, 55%). [$α$]$_D^{20}$ = +121 (c = 0.2, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): $δ$ = 5.46 (dd, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, 1 H, 3-H), 5.11 (dd, $J_{4,5}$ = 9.6 Hz, 1 H, 4-H), 4.94 (d, $J_{1,2}$ = 3.7 Hz, 1 H, 1-H), 4.86 (dd, 1 H, 2-H), 3.86 (m, 1 H, 5-H), 3.54–3.36 (m, 6 H, 6a-, 6b-H, CH$_2$CH_2O, CH$_2$CH_2N$_3$), 3.40 (s, 3 H, CH_3O), 2.69 (m, 2 H, CH$_3$COCH_2CH$_2$), 2.48 (m, 2 H, CH$_3$COCH$_2$CH_2), 2.15 (s, 3 H, CH_3Lev), 2.04, 2.02 (2 × s, 3 H each, CH_3CO), 1.83 (m, 2 H, OCH$_2$CH_2CH$_2$N$_3$) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): $δ$ = 171.4, 170.4, 170.2 (CO), 96.9 (C-1), 71.0 (C-2), 70.1 (C-3), 69.0 (CH_2), 68.9, 68.5, 68.4 (C-4, -5, CH_2), 55.5 (CH$_3$O), 48.4 (CH$_2$N$_3$), 37.8 (CH$_3$COCH$_2$CH$_2$), 29.8 (CH$_3$Lev), 29.2 (OCH$_2$CH$_2$CH$_2$N$_3$), 27.9 (CH$_3$COCH$_2$$CH_2$), 20.8 ($CH_3$CO) ppm. HRMS: m/z: found 482.1765; $C_{19}H_{29}N_3NaO_{10}$ requires 482.1751 [M + Na]$^+$.

1-(2′,3′,4′,6′-Tetra-O-acetyl-α-D-mannopyranosyloxyethyl)-4-{3-(methyl 2′′,3′′-di-O-acetyl-6′′-O-levulinyl-α-D-glucopyranoside)-propyl}-1H-1,2,3-triazole (24): Flash chromatography (EtOAc). [$α$]$_D^{20}$ = +56 (c = 0.3, CH$_2$Cl$_2$). ^{1}H NMR (300 MHz, CDCl$_3$): $δ$ = 7.4 (brs, 1 H, CHN), 5.48 (dd, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, 1 H, 3-H), 5.27–5.14 (m, 5 H, 4-, 2′-, 3′-, 4′-H), 4.95 (d, $J_{1,2}$ = 3.6 Hz, 1 H, H-1), 4.88 (dd, 1 H, 2-H), 4.82 (brs, $J_{1′,2′}$ < 1 Hz, 1 H, H-1′), 4.47 (m, 2 H, CH$_2$), 4.24 (m, 1 H, 6a′-H), 4.09 (m, 2 H, 5′-, 6′b-H), 3.90–3.40 (m, 9 H, 5-, 6a-, 6b-H, NCH$_2$CH$_2$CH_2O, NCH$_2$CH$_2$CH$_2$O, OCH_2CH$_2$), 3.36 (s, 3 H, CH_3O), 3.02 (m, 2 H, OCH$_2$CH_2), 2.70 (m, 2 H, CH$_3$COCH_2CH$_2$), 2.49 (m, 2 H, CH$_3$COCH$_2$CH_2), 2.15 (s, 3 H, CH_3Lev), 2.14, 2.12, 2.09, 2.06, 2.03, 1.98 (6 × s, 3 H each, 6 × CH_3CO), 1.91 (m, 2 H, OCH$_2$CH_2CH$_2$N) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): $δ$ = 171.6, 170.8, 170.2, 169.8 (CO), 144.0 (NC=CH), 122.6 (NC=CH), 97.6 (C-1′), 96.9 (C-1), 71.0 (C-2), 70.2 (C-3), 69.6, 69.2, 68.8, 68.5, 66.1 (C-4, -5, -2′, -3′, -4′, -5′, CH$_2$), 67.9, 67.0 (2CH$_2$), 62.5 (C-6′), 55.5 (CH$_3$O), 37.8 (CH$_3$COCH$_2$CH$_2$), 30.4 (NCH$_2$CH$_2$), 29.8 (CH$_3$Lev), 28.0 (CH$_3$COCH$_2$$CH_2$), 26.1 (N$CCH_2$), 21.0 ($CH_3$CO) ppm. HRMS: m/z: found 882.3087, $C_{37}H_{53}N_3NaO_{20}$ requires 882.3120 [M + Na]$^+$.

Acknowledgments

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